FEEDING OF NAUPLIAR AND ADULT CARNIVOROUS CYCLOPOIDS (CRUSTACEA: COPEPODA)

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FEEDING OF NAUPLIAR AND ADULT CARNIVOROUS CYCLOPOIDS (CRUSTACEA: COPEPODA)

An abstract of a Thesis by
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The problem. Little is known about the feeding of naupliar cyclopoid copepods, though they are often abundant in the zooplankton. The objective of the current study is to compare feeding of naupliar and adult cyclopoids by testing them on prey types they commonly encounter.

Procedure. In-laboratory feeding experiments, using prey difference counts, radiolabeled prey, and predator survivorship, were carried out on a variety of prey types. Predator species included Cyclops bicuspidatus thomasi, Mesocyclops edax, and Cyclops vernalis.

Findings. Adult cyclopoids predated on cyclopoid nauplii, Bosmina, Asplanchna, and Paramecium, but not on Ceriodaphnia, ostracods, Selenastrum, nor bacteria. One-day old nauplii fed on bacteria mixed with diatomaceous earth and dissolved organics, but not on Ceriodaphnia, Bosmina, Asplanchna, Paramecium, Selenastrum, nor free bacteria. The prey difference method worked well for detecting predation of forms larger than 150 μ, while the survivorship method was useful for testing bacteria and algae. The experiment with radiolabeled prey worked poorly.

Conclusions. Carnivorous cyclopoid adults and their nauplii differ in food habits, so they do not compete for this resource. Predation by adults is selective against small bodied animals, and is capable of influencing zooplankton community structure, while nauplii have little effect. Cannibalism can regulate cyclopoid populations. Neither adults nor nauplii survive very long on unispecific cultures.

Recommendations. Future studies should concentrate on comparing the feeding of cyclopoid stages NI-NIII, NIV-NV and NVI-CIII on bacteria-coated detritus (known size fractions), algae, and dissolved organics. The best methods to use for these foods are predator survivorship, and radiolabeled prey, with modifications in the method of the current study.

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INTRODUCTION

Copepods, together with cladocera and rotifers, are the most important members of the freshwater zooplankton, both in total biomass and rates of energy transfer (Wetzel, 1975). Copepods are distributed worldwide and are found in a variety of habitats, ranging from the littoral zone of ponds to the open waters of lakes. In marine systems, copepods are the dominant planktonic group, not only in numbers, but also in species diversity. Marshall (1973) believes copepods are "perhaps the most numerous animals in the world".

The study of zooplankton feeding interactions is important; first, because zooplankton constitute the major link between algae and fish production. Knowledge of trophic structure can reveal information useful to practical problems in fish production (Hillbricht-Ilkowska et al., 1975). Second, zooplankton predator prey systems serve as effective models for investigating problems in theoretical ecology, such as the role of predation in structuring natural communities. The zooplankton community is a good model because population densities are frequently high plus hiding places are limited; thus, predation pressures can be fierce. Kerfoot (1977a) discovered predaceous copepods influenced the evolution of a population of Bosmina by selecting against individuals lacking effective morphological

and/or behavioral defenses. Third, studies on feeding within the zooplankton community can reveal useful information about the anatomy and physiology of the animals involved. For example, Friedman and Strickler's (1975) discovery of chemoreceptors in calanoid copepods arose from investigations of feeding habits. Finally, marine copepods have been observed ingesting oil droplets (Conover, 1971). They may be significant in converting oil spills to detritus, which can readily be decomposed in the sediments.

The free living copepods are primary and secondary consumers. A typical food web for freshwater ponds and lakes is illustrated in Figure 1. Calanoid copepods, which dwell in open waters, are herbivorous, though on occasion they can switch to a carnivorous mode (Marshall, 1973). In the sea, these filter feeders serve as the main link between algae and planktivorous fish. The cyclopoid copepods, on the other hand, are omnivorous. Most species will capture both plant and animal food, though some are strict vegetarians and some strict carnivores (Fryer, 1957b). Cyclopoid copepods are common in littoral and limnetic regions of ponds and lakes (Yeatman, 1959) and less common in marine environments, which are dominated by the calanoids.

Numerous studies have been conducted to determine food items copepods capture, ingest, and assimilate. A variety of methods have been attempted. Examination of gut

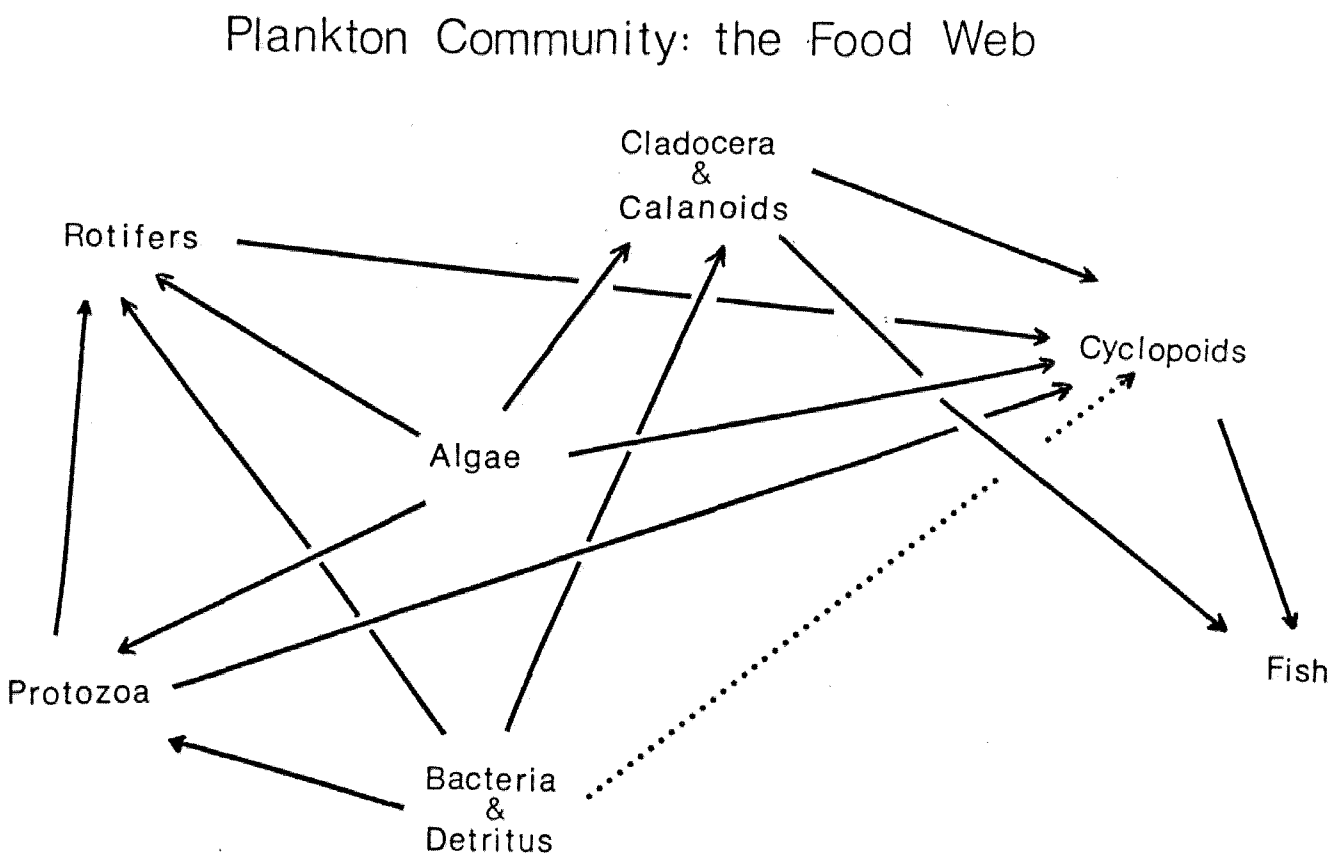

Figure 1. A freshwater grazing food web. Arrows point from food source to the animal feeding on that food. Solid lines represent known relationships; the dotted line is a conjectured relationship. Note: feeding on young age classes is not considered, nor is input to detritus.

contents (Fryer, 1957b) and fecal pellets (Marshall and Orr, 1955) from animals sampled in the field can provide direct evidence of prey captured. However, these techniques are biased in favor of detecting hard-bodied prey. The remains of soft-bodied animals, such as flagellates and rotifers, are often impossible to classify and have been described as "indeterminant mush" (Fryer, 1957b). The survivorship method has been used by several workers to define food requirements for copepods reared in laboratory (Gehrs and Robertson, 1975; Martin and Novotny, 1975; Provasoli et al., 1970; Smyly, 1970). Negative results do not prove a failure to feed, however, nor can feeding rates be calculated. The "prey difference" method examines the effect on prey populations of introducing a predaceous copepod. This method has been used frequently (Anderson, 1970; Brandl and Fernando, 1978; Confer, 1971; Dodson, 1975; Kerfoot, 1977a, 1977b, 1978; McQueen, 1969; Paffenhöfer, 1971) and can be used to calculate feeding rates. The "predator gain" method measures predation by uptake of radiolabelled food and has been used to detect feeding rates (Marshall and Orr, 1955, 1956; Monakov and Sorokin, 1960), but presents some problems (Conover and Francis, 1973). For carnivores, direct observation may be most reliable (Li and Li, 1979) and serves the added benefit of describing predator and prey behavior. Information supporting feeding studies comes from zooplankton distribution (Patalas, 1975),

mouthpart anatomy (Fryer, 1957a), digestive enzymes (Hasler, 1937), and anatomical descriptions of sensory receptors associated with prey detection and handling (Friedman and Strickler, 1975; Strickler, 1975a).

Most research on the feeding of carnivorous cyclopoid copepods has centered on their predation of other crustacean zooplankters (Anderson, 1970; Brandl and Fernando, 1978; Confer, 1971; Dodson, 1975; Kerfoot, 1977a, 1977b, 1978; Li and Li, 1979; and McQueen, 1969). Other investigated prey include algae and insect larvae (Fryer, 1957b), bacteria (Monakov and Sorokin, 1960), rotifers (Li and Li, 1979), protozoa (Coker, 1933, in Fryer, 1957b; Martin and Novotny, 1975) and fish fry (Davis, 1959).

Cyclops bicuspidatus thomasi Forbes, Mesocyclops edax Forbes, and Cyclops vernalis Fischer are all widely distributed North American cyclopoids, often occurring in great numbers (Yeatman, 1959).

Several researchers have examined the feeding of C. b. thomasi. Martin and Novotny (1975) found they could be readily cultured on a diet of non-axenic Paramecium, but cautioned that adult stages readily cannibalize. Cannibalism was also reported by McQueen (1969), but not observed by Anderson (1970). C. b. thomasi predates heavily on Bosmina (Kerfoot, 1978) and is also known to attack fish fry (Davis, 1959).

M. edax has been shown to feed on mixed protozoan

cultures (Brandl and Fernando, 1975), young Ceriodaphnia (Brandl and Fernando, 1975), naupliar and copepodid stages of Diaptomus (Confer, 1971), naupliar M. edax (Brandl and Fernando, 1975), and fish fry (Davis, 1959).

C. vernalis, the largest of the three cyclopoids considered here, has been used frequently in feeding studies. This species has been reared successfully on mixed protozoan cultures (Coker, 1933, in Fryer, 1957b; Brandl and Fernando, 1975). Gut contents of C. vernalis collected in the field revealed remains from chydorid cladocerans and unidentified cyclopoid copepodids (Fryer, 1957b). C. vernalis has also been found to predate on rotifers (Fryer, 1957b; Li and Li, 1979), Bosmina (Kerfoot, 1978; Li and Li, 1979), Ceriodaphnia neonates (Brandl and Fernando, 1975; Li and Li, 1979) but not Ceriodaphnia adults (Li and Li, 1979), and naupliar stages of C. vernalis (Loundes, 1928, in Fryer, 1957b; Robertson et al., 1974). Dodson (1975) also detected cannibalism, but at very low rates.

The copepods exhibit two types of feeding mechanisms, filter feeding and raptorial capture, which are described in detail by Marshall (1973) and Fryer (1957a). Filter feeding is effective for trapping large numbers of tiny food items such as algal cells, while raptorial (grasping) capture is used for feeding on large prey. Usually a given copepod species will specialize in one mode

or the other. For example, Calanus finmarchicus spends most of its feeding efforts filtering diatoms while Tortanus discaudatus is a voracious predator on pelagic crustacea. Each can, however, switch over to the other feeding mode should food conditions dictate (Marshall, 1973).

Filter feeding copepods are restricted to feeding on particles within certain size limits. The lower limit is determined by the distance between the setules on the filter. Marshall and Orr (1955), working with Calanus and a variety of algal species, found that cells less than 10 μ in diameter were captured inefficiently and those less than 5 μ were rarely caught. This would preclude the capture of most free bacteria, which are predominately in the 0.5 to 5 μ size range. Much of the bacteria found in aquatic environments is adsorbed to detritial particles (Baker and Bradham, 1976), so the actual size of bacteria-coated particles may be above this critical lower limit. Some invertebrates specialize in this food resource (Marzolf, 1966; Calow, 1974). Cells larger than 50 μ can not be effectively filtered; rather, they can be captured raptorially, (Conover, 1968), though this process is mutually exclusive to filter feeding.

Carnivorous cyclopoid copepods specialize in raptorial capture, where large particles (plant, animal, detritus, etc.) are seized individually and manipulated by

the mouthparts. The prey item may be ingested whole or fragmented, then ingested. Figure 2 (top) illustrates the important appendages used in feeding by the adult cyclopoid. Raptorial feeding requires different tools and behavior than filter feeding. The prey are often motile and large. While filter feeders are equipped with fine setae on their maxilla, the carnivorous copepod has fewer, but stronger, setae used for grasping and impaling. The cyclopoid is equipped with sharp barbs on the mandibular gnathobases which allow the animal to tear and slice its prey. In the herbivore, the gnathobases are blunt, which makes them suitable for crushing.

Once captured, the prey is consumed quickly. With a rapid movement (kick by the urosome plus the 2nd, 3rd, and 4th thoracic appendages), the prey is seized and pierced by the maxillules (1st maxilla), covered by the 2nd maxilla and maxillipeds, and masticated by the mandibular gnathobases (Fryer, 1957b). Soft body parts are quickly ingested and remains discarded.

Previously, it was assumed that encounters between cyclopoids and their prey occurred by chance (Fryer, 1957a) and the predator only reacted when touched. But Kerfoot (1978) described hunting maneuvers which are far from random. Purposeful movements are made possible through the presence of vibration receptors located on the antennae and body proper. These allow the animal to be

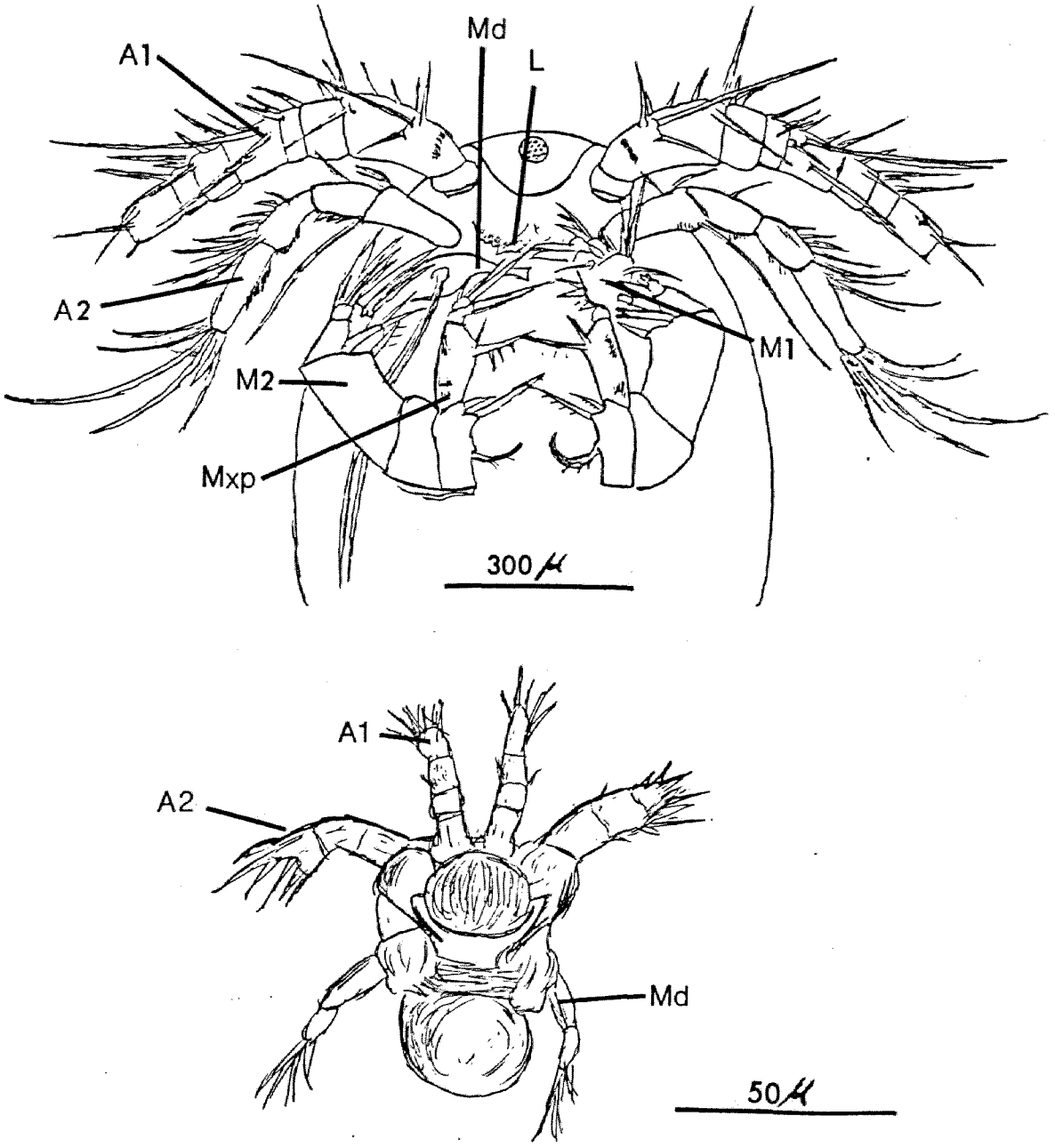

Fig. 2. Top. Mouthpart anatomy of adult Macrocyclops albidus (after Fryer, 1957a). The distal extremities of the 2nd maxilla and maxilliped are removed, revealing the left maxillule. The right maxillule and left mandible are not shown. Only the 1st 7 (of 17) segments of the 1st antennae are shown. Symbols used: A1-1st antennae, A2-2nd antennae, Md-mandible, M1-1st maxilla (maxillule), M2-2nd maxilla, Mxp-maxilliped, L-labrum. Btm. Nauplius I stage of Mesocyclops edax showing the ventral surface.

repelled by larger animals, producing vibrations of large magnitude, and to be attracted to smaller animals, which produce vibrations of a small magnitude (Strickler, 1975a). Long distance chemotaxis may also occur in the copepods (Elbourn, 1966; Katona, 1973), allowing the animal to respond from as far away as 20 body lengths. Food selection does not require the activity of photoreceptors, since it can occur quite well in the dark, according to Richman and Rogers (1969). Cyclopoid copepods are now described as examples of cruising predators (Gerritson and Strickler, 1977), animals which move around in search of prey, can swim quickly and efficiently when necessary, and are responsive to sensations generated by the prey.

Recently, morphological and behavioral traits of cladocera who were either successful or unsuccessful in avoiding copepod predation have been described (Kerfoot, 1977a, 1977b, 1978; Li and Li, 1979). These traits include size, swimming speed, escape movements, the "dead man's response", carapace characteristics, and life stage. When nearby motile prey are detected by carnivorous copepods, the predator must "key in" to the source of the disturbances in order to complete the encounter. Should these disturbances stop, the predator is left confused and often fails to make contact with the prey. Herein lies the importance of the "dead man's response", evolved by the slow moving Bosmina (Li and Li, 1979). B. longirostris populations

subject to heavy cyclopoid predation use this tactic quite successfully (Kerfoot, 1978).

Copepods may ingest or discard prey they capture. Manipulation of the mouthparts allow the animals to select on the basis of taste; worthless or toxic items can be rejected and useful foods ingested. Friedman and Strickler (1975) report that the calanoid copepod, Diaptomus pallidus, has chemoreceptors on all of its mouthparts. Conover (1966; in Friedman and Strickler, 1975) observed when copepods were offered their own fecal pellets as food, the animals tore open the peritrophic membrane, manipulated the particles with their mouthparts, then rejected the waste as unsatisfactory. Copepods do make mistakes though. Some algae, regularly ingested, pass through the digestive tract unharmed, providing no nutritious benefit to the predator (Fryer, 1957b). Apparently, filter feeding copepods accept food particles a priori if no cause for rejection is detected. Paffenhöfer and Strickland (1970) demonstrated that copepods will ingest inert plastic pellets.

The larval stage of cyclopoid copepods, the nauplius, is both motile and free living. Copepods molt through five or six naupliar stages and five copepodid (or juvenile) stages before achieving the adult form (Yeatman, 1959). Herein, these developmental stages will be referred to as NI, NII,...,NVI; and CI, CII,..., CV, respectively. Figure 2 (bottom) illustrates the morphology of the copepod

nauplius (NI stage) which bears three pair of appendages, the 1st and 2nd antennae and the mandibles.

As the nauplius molts, the body becomes larger and more cylindrical in shape. The two pair of maxilla, the thoracic body segments, and the first two thoracic appendages are added during the nauplius instars. During copepodid development, the last four thoracic appendages are added along with a segmented abdomen. The entire developmental time for cyclopoid copepods may range from seven to 180 days (Pennak, 1978), most commonly 21 to 35 days.

Studies of copepod feeding have dealt primarily with late copepodid and adult instars. However, the feeding of the younger stages is also important. Copepod nauplii and early copepodids sometimes carry greater biomass (Cushing and Vecetic, 1963) and can play a major role in the consumption of marine phytoplankton (Paffenhöffer, 1971). Feeding of the nauplius is incompletely understood, and statements in the literature are sometimes contradictory. For example, Dodson (1975) states, "All predaceous freshwater crustacean zooplankton are herbivorous as nauplii...", while Marshall (1973), in her review article on copepod feeding, says "The larvae of carnivorous copepods are sometimes herbivorous, but are more often non-feeding, or carnivorous like their parents". Few studies have investigated these early stages in detail (Bosselmann, 1975;

Gauld, 1959; Marshall and Orr, 1956; Monakov and Sorokin, 1960; Paffenhöfer, 1971).

Monakov and Sorokin (1960), using ^{14}C as a tracer, determined that cyclopoid nauplii could assimilate bacteria. They calculated that the quantity assimilated would provide enough energy to sustain normal growth and development. This short term study did not test if nauplii actually do grow and develop when fed only bacteria. Provasoli et al. (1959) suggest that bacteria alone would not be sufficient. Methods for culturing copepods (Martin and Novotny, 1975) call for using protozoan cultures, rich in bacteria, yeast, and dried plant remains, providing a rich variety of foods for the developing stages. Nauwerck (1963, in Bosselmann, 1975) suggests that bacteria may be an important source of nutrition to copepod nauplii, especially during the bacterial decomposition following plankton blooms.

Feeding must be a difficult job for the nauplius, since the mouthparts are so poorly developed. Those cyclopoids that do feed use the two pair of antennae for propulsion, and mandibles for food collection (Gauld, 1959). Setae on the latter appendages form a "scoop net". Though some nauplii catch and ingest large algal cells, others do not feed at all (Gauld, 1959). Early naupliar (NI-II) stages of Calanus do not feed, while later stages (NIII-VI) filter diatoms and small flagellates (Marshall and Orr, 1956). These later stages have better developed mouthparts;

spines on the coxa and basipod of the antennae are more fully developed and the second maxilla (the main filtering appendages in adults) are present, though still probably not functional.

The objective of my research is to compare the feeding abilities of cyclopoid adults and nauplii by testing them on prey types they commonly encounter. Laboratory feeding experiments will test species of carnivorous cyclopoids on bacteria, algae, protozoa, rotifers, cladocera, ostracods, and naupliar cyclopoids. Results of these feeding trials will be used to clarify the role of naupliar and adult cyclopoids in the zooplankton community.

MATERIALS AND METHODS

Predator. Cyclopoid copepods were obtained from Witmer pond (34th St., Des Moines, IA) with a #20 plankton net (pore size = 110 μ) and returned to the laboratory. There, the animals were isolated, identified to species according to Yeatman (1959), and cultured in gallon jars by the method of Martin and Novotny (1975). Species identified were Cyclops bicuspidatus thomasi, Mesocyclops edax, and Cyclops vernalis. These species correspond to the predominant cyclopoid for the summer of 1977, 1978, and 1979, respectively.

Cyclopoid adults were needed in large numbers for

the feeding experiments. Various methods for effecting rapid isolation, including the use of anaesthetics (Straškraba, 1964) were tested. These techniques were inefficient or too time consuming, so a procedure was developed that worked for harvesting cyclopoids in large numbers. The plankton concentrate plus an equal volume of culture medium were delivered to a separatory funnel and allowed to remain for one to two days. After this time period, cyclopoid adults and late copepodids predominated, presumably because other members of the plankton suffered high mortality. After the waiting period, the detritus was released from the bottom of the funnel, algae siphoned off the top, and both were discarded. The remaining suspension was passed through a 240 μ filter, which concentrated cyclopoids (and large cladocera, if present). The filter contents were then washed onto spot plates, where the cyclopoids could be readily removed with a mouth pipette. Cyclopoid nauplii were obtained by hatching eggs from known species in the laboratory. These animals were used in experiments within the first day after hatching.

Prey. A variety of prey types were used in feeding experiments. These included representatives of the bacteria, algae, protozoa, rotifers, ostracods, large and small cladocera, and cyclopoid copepods. Since isolation and culture differs among them, each is described separately.

The bacteria, Aeromonas hydrophilia (Pseudomonidaceae)

and Enterobacter cloacae (Enterobacteriaceae), were taken from Witmer Pond water and isolated by streaking on Tryptic soy Agar (Difco) plates. Commonly occurring colonies were subcultured in Nutrient Broth (Difco) and identified through biochemical tests (API 20E System, Analytab Products). Stock cultures were kept refrigerated and regularly subcultured.

The algae, Selenastrum capricornutum (Chlorophyta) was obtained from stock cultures, originally purchased from the University of Texas, and maintained by the method of Bartsch (1971).

Paramecium multimicronucleatum (Protozoa, Ciliata), though a common pond inhabitant, was not isolated. Rather, it was taken from stock cultures maintained in the Drake University Biology Department and used to innoculate cultures maintained by the methods of Martin and Novotny (1975). This culture, though dominated by P. multimicronucleatum, also contained a variety of flagellates, bacteria, yeast, plant detritus (derived from added Cerophyl, Cerophyl Laboratories, Kansas City, MO), and dissolved organics. Before use in feeding experiments, paramecia were centrifuged and washed three times, resuspended in salt solution, mixed in a beaker with a magnestir, and counted in triplicate, using a Sedgewick-Rafter chamber under the microscope. Appropriate volumes were added to each experimental vessel to give an initial count of 207/feeding

chamber (10 ml volume).

Asplanchna sp. (Rotatoria, Monogononta), Ceriodaphnia sp. (Crustacea, Cladocera), and Bosmina coregoni (Crustacea, Cladocera) were isolated from plankton hauls in a similar manner to that described for the cyclopoids. Since isolation was rapid and culturing not always successful, neither the cladocera nor the rotifers were maintained in laboratory cultures. Instead, they were used in experiments shortly after isolation. Brooks (1959) was used for identification of the cladocera while Needham and Needham (1962) and Meglitsch (1972) were used for the rotifers.

Five to ten individuals of each predator and prey species were measured using a calibrated ocular micrometer. The one exception was bacteria, which were too small for accurate measurement. Instead, their dimensions were obtained from Bergey's Manual (Breed et al., 1957). These measurements are listed in Table 1.

Feeding trials. Feeding experiments were conducted in the laboratory, within glass dishes at 20°C. and a diurnal light/dark cycle of 12 hours diffuse light:12 hours dark. Experiments lasting less than 12 hours were run during the light phase. Most trials were in six cm diameter watch glasses, containing 20 ml growth medium. Exceptions were the predator survivorship studies, where adults were maintained in 10 ml beakers and nauplii in depressions of one ml volume (spot plates). Unless stated

Table 1. Mean sizes of predators and prey. On crustacea, measurement includes appendages but not setae. All predators are adult females unless specified otherwise.

organism	mean dimensions (length by width in μ)
Predators:	
Cyclops bicuspidatus thomasi	1025 X 385
Cyclops bicuspidatus thomasi nauplii	163 X 125
Mesocyclops edax	1180 X 360
Cyclops vernalis	1240 X 390
Cyclops vernalis nauplii	145 X 135
Prey:	
Aeromonas hydrophilia	1.3 X 0.6
Selenastrum capricornutum	26 X 4.0
algae (unidentified)	22 X 22
Paramecium multimicronucleatum	180 X 55
Asplanchna sp.	458 X 394
Bosmina coregoni	310 X 280
ostracods (unidentified)	550 X 330
Ceriodaphnia sp.	1590 X 890

otherwise, "growth medium" refers to a solution of inorganic salts ($MgSO_4$, $NaHCO_3$, $KHCO_3$, $CaCl_2$, K_2HPO_4, and Na_2SiO_3) suggested for use in crustacean cultures by Sheer and Armitage (1973, in Martin and Novotny, 1975). This medium was used for all handling of animals, feeding trials, copepod and cladoceran cultures, and suspension of bacterial feed. After mixing, the growth medium was sterilized and freed of particulates through Millipore filtration (0.45 μ pore size).

Paramecium, Asplanchna, Bosmina, Ceriodaphnia, and cyclopoid nauplii were counted in a ruled dish under a dissecting microscope. Algae and bacteria were counted under a phase contrast microscope using a hemacytometer and Petroff-Hauser Cell, respectively. Pour plates were also used to estimate bacterial numbers. This method, which counts only viable cells, produced values about one order of magnitude less than the direct counts.

Prey Difference Method. Three different methods were used to detect predation. Most common was the "prey difference method", where prey numbers were counted before and after contact with the predator. Controls consisted of a similar density of prey, without the predator present, which were counted before and after the same time interval as experimentals. The "prey difference method" was conveniently used for trials employing the larger prey organisms, but was less successful for testing bacteria

and algae.

Species of predators used in prey difference trials were as follows: Adult C. b. thomasi were tested for feeding on Paramecium, ostracods, Ceriodaphnia, and C. b. thomasi nauplii. Adult and naupliar C. vernalis were tested on Asplanchna and Bosmina. Naupliar C. b. thomasi were tested for feeding on Aeromonas, an unidentified alga, Paramecium, and Ceriodaphnia.

Data from the "prey difference" trials were used to generate a relative difference (d) which is equal to:

$$d = \frac{N_t - N_o}{N_o}$$

where N_o equals the initial density of prey and N_t equals the final density. This ratio usually takes on a value between zero and negative one. Positive values can occur when the prey population shows an increase. Values of "d" for control and experimental groups were compared and significance established through the Mann-Whitney U-test (Sokal and Rohlf, 1969).

Predator Gain Method. The "predator gain method" consisted of measuring uptake of radioactivity by the predator (M. edax adults) after contact with tagged prey (bacteria grown in the presence of ^{32}P). Details of this procedure are presented in the discussion section of this thesis.

Survivorship Method. The third method for detecting predation was the "survivorship method", which compared the number of days lived by predators receiving food as opposed to controls not receiving food. The predators (C. vernalis adults and nauplii) were isolated, one per container, and transferred daily into new containers with sterile media. Each day experimental groups received one of the following food sources: bacteria at a final density of 10^7 cells/ml, Selenastrum at a final density of 10^5 cells/ml, or a 1:10 dilution of filtrate (0.45 μ filter) from the mixed protozoan culture described previously. Bacteria plus diatomaceous earth (particle size = 2-20 μ) was also tested. All animals were checked daily for response to touch from a probe. The survival time for individuals from each group was compared by the Mann-Whitney U-test. The survivorship method is more comprehensive than the previous two methods described. For the predator to avoid starvation, it must not only be able to consume the prey item, but must also be able to assimilate it.

After high mortality rates were observed in both experimental and control groups, a second control was started to see if deaths were due to starvation or to trauma from the daily manipulations. This group received an optimum diet of the protozoan culture previously described. This mixture provides adequate nourishment to

both nauplii and adults for growth and development (Martin and Novotny, 1975).

RESULTS

Prey Difference Trials. The prey difference method was useful for measuring cyclopoid predation on all prey types except bacteria and algae. Procedural problems with the latter two caused inconclusive results. Further study of predation on bacteria was done using the "predator gain" and survivorship methods. Raw data for all feeding experiments is presented in the Appendix (Table 12).

Table 2 summarizes each feeding experiment, indicating the number of replicates, duration, means of initial and final counts, and means of absolute differences in prey numbers. The prey difference statistic was generated by subtracting initial prey counts from final live prey counts. This represents the total mortality in the vessel, regardless of whether the prey individuals were removed (eaten) or not. One exception to this occurred in the algae experiment where dead individuals could not be distinguished from live, so "final live" counts actually represent the sum of live and dead cells.

All large prey animals (Asplanchna, Ceriodaphnia, ostracods, Bosmina, and cyclopoid nauplii) were individually pipetted into the experimental chambers, so initial counts are exact. Small prey (Paramecium, algae, and bacteria)

Table 2. Cyclopoid predation on selected prey types determined by the prey difference method. Mean prey numbers ± one standard deviation are indicated. Symbols used are: "n" for number of replicates, "t" for duration of the feeding period (hours), and "nc" for not counted. The predator for experiments with Paramecium, ostracods, Ceriodaphnia, nauplii, algae, and bacteria was Cyclops bicuspidatus thomasi. The predator for experiments with Asplanchna and Bosmina was Cyclops vernalis. Nauplii of C. b. thomasi were also tested for feeding on bacteria and algae. Only mature stages of the prey were used unless indicated otherwise.

situation	n	t	initial count	final counts		final live minus initial count
				dead	alive	
Paramecium only	5	3	70±1.91	nc	66.4±4.72	-3.6±4.72
Paramecium plus nauplius	5	3	70±1.91	nc	73.8±6.84	3.8±6.84
Paramecium plus adult	5	3	70±1.91	nc	41.6±7.16	-28.4±7.16
Asplanchna only	5	12	20	0.8±0.83	28.0±4.29	8.0±4.29
Asplanchna plus nauplius	5	12	20	1.4±1.14	27.4±3.64	7.4±3.64
Asplanchna plus adult	5	12	20	0.8±1.30	19.8±3.11	-0.2±3.11
Ostracods only	5	24	10	0	10	0
Ostracods plus adult	5	24	10	1.8±1.48	9.2±0.83	-0.8±0.83

Table 2 (continued)

situation	n	t	initial count	final counts		final live minus initial count
				dead	alive	
Bosmina only	5	48	20	13.6±1.50	5.6±1.68	-14.4±1.68
Bosmina plus nauplius	5	48	20	17.4±3.35	4.4±1.52	-15.6±1.52
Bosmina plus adult	5	48	20	3.4±2.30	1.2±1.63	-18.8±1.63
Bosmina only	5	12	20	3.6±5.93	19.0±2.35	-1.0±2.35
Bosmina plus nauplius	5	12	20	1.8±1.10	18.6±2.77	-1.2±2.77
Bosmina plus adult	5	12	20	2.6±2.08	16.4±2.95	-3.6±2.95
Ceriodaphnia only	8	27	9.8±1.13	0.8±2.21	11.1±4.38	1.4±3.39
Ceriodaphnia plus nauplius	5	27	9.8±0.98	0.8±0.83	7.4±0.89	-1.6±0.89
Ceriodaphnia plus adult	8	27	9.6±0.74	0.5±0.76	8.6±3.82	-1.0±3.28
Nauplii only	5	20	20.2±.83	0.2±0.45	18.8±0.83	-1.4±1.14
Nauplii plus adult	5	20	19.6±.89	0.6±1.34	7.0±3.64	-12.6±3.64

Table 2 (continued)

situation	n	t	initial count	final counts		final live minus initial count
				dead	alive	
Algae only	5	6	603±196.1	nc	402±67.5	-201±230.8
Algae plus 1 nauplius	5	6	603±196.1	nc	552±119.4	-51±225.2
Algae plus 5 nauplii	5	6	603±196.1	nc	466±86.8	-138±186.3
Bacteria only	5	4	nc	nc	822±96.6	-
Bacteria plus nauplius	5	4	nc	nc	959±321.5	-

were subsampled from a precounted feed suspension, so some indeterminant error is contained in these initial counts.

Data from the Ceriodaphnia experiment represents two experiments pooled; valid since both used the same procedure. The Bosmina experiments could not be pooled because each lasted different lengths of time. Contact time between predator and prey is an important variable influencing their interaction.

The prey difference method was also used to detect naupliar grazing on bacteria (Enterobacter cloacae). Bacterial counts used pour plates to estimate the number of live bacterial cells. Initial counts of the bacterial feed suspension were attempted, but bungled, so this data is missing from Table 2. Equal numbers of bacteria were introduced to all vessels, so both controls and experimentals had similar starting concentrations. The data indicate the presence of the nauplii caused an increase in bacteria, rather than a decrease due to grazing. However, this increase was not statistically significant.

An unidentified alga was tested for naupliar feeding using the prey difference method. The size of individual cells (22 μ) was within the range of particle sizes readily grasped by small copepods (Marshall, 1973). The nauplius had little or no effect on numbers of this algal species (Table 2). The algae counts also show high variability. These results and the bacterial pilot trials showed that

the prey difference technique was not the best way to detect grazing on such small organisms.

Table 3 lists the Mann-Whitney U-test statistic and significance level for each feeding experiment employing the prey difference method. Predation is indicated when the one-tailed significance falls below 0.05. An overview of this table shows that adult C. b. thomasi predated on Paramecium and C. b. thomasi nauplii, but not on Ceriodaphnia nor ostracods. Adult C. vernalis predated on Asplanchna and Bosmina (48 hour experiment). The second Bosmina experiment, lasting 12 hours, did not indicate significant predation. Nauplii (C. b. thomasi tested on bacteria, unknown algae, Paramecium, and Ceriodaphnia; and C. vernalis tested on Asplanchna and Bosmina) did not feed on any of the prey offered. Results confirm preliminary observations where adults were observed responding to and attacking Paramecium, Asplanchna, and Ceriodaphnia. Attacks on Ceriodaphnia were never successful, though on one occasion a Ceriodaphnia lost an antennae. This attack behavior was never seen when the nauplius was placed with these prey. The only response which the nauplius made was to escape, even when in contact with Paramecium.

Results from the Paramecium and Ceriodaphnia experiments (Figures 3 and 4) show significant and no significant predation, respectively. C. b. thomasi adults clearly feed on Paramecium, as is indicated by the high mortality of

Table 3. Statistical significance of data from prey difference trials. The relative difference in prey numbers $((N_t - N_0) \div N_0)$ for experimentals and controls are compared to produce the Mann-Whitney U-test statistic U_s, which is used to establish significance. If the one tailed significance is less than 0.05, the null hypothesis is rejected and predation is indicated. "Cbt" refers to cyclopoids of the species Cyclops bicuspidatus thomasi and "Cv" refers to cyclopoids of the species Cyclops vernalis.

		Nauplius		Adult	
Prey	Predator	U_s	Signif. ($p \leq x$)	U_s	Signif. ($p \leq x$)
Bacteria	Cbt	16	.50	--	--
Unknown algae (1 pred/vessel)	Cbt	20	.10	--	--
Unknown algae (5 pred/vessel)	Cbt	15	.50	--	--
Paramecium	Cbt	22.5	.10	25	.01
Asplanchna	Cv	13.5	.50	23.5	.05
Bosmina (48 hr contact)	Cv	18	.50	24	.05
Bosmina (12 hr contact)	Cv	13	.50	18	.50
Cyclopoid nauplii (Cbt)	Cbt	--	--	25	.01
Ostracods	Cbt	--	--	20	.50
Ceriodaphnia	Cbt	33	.10	43.5	.50

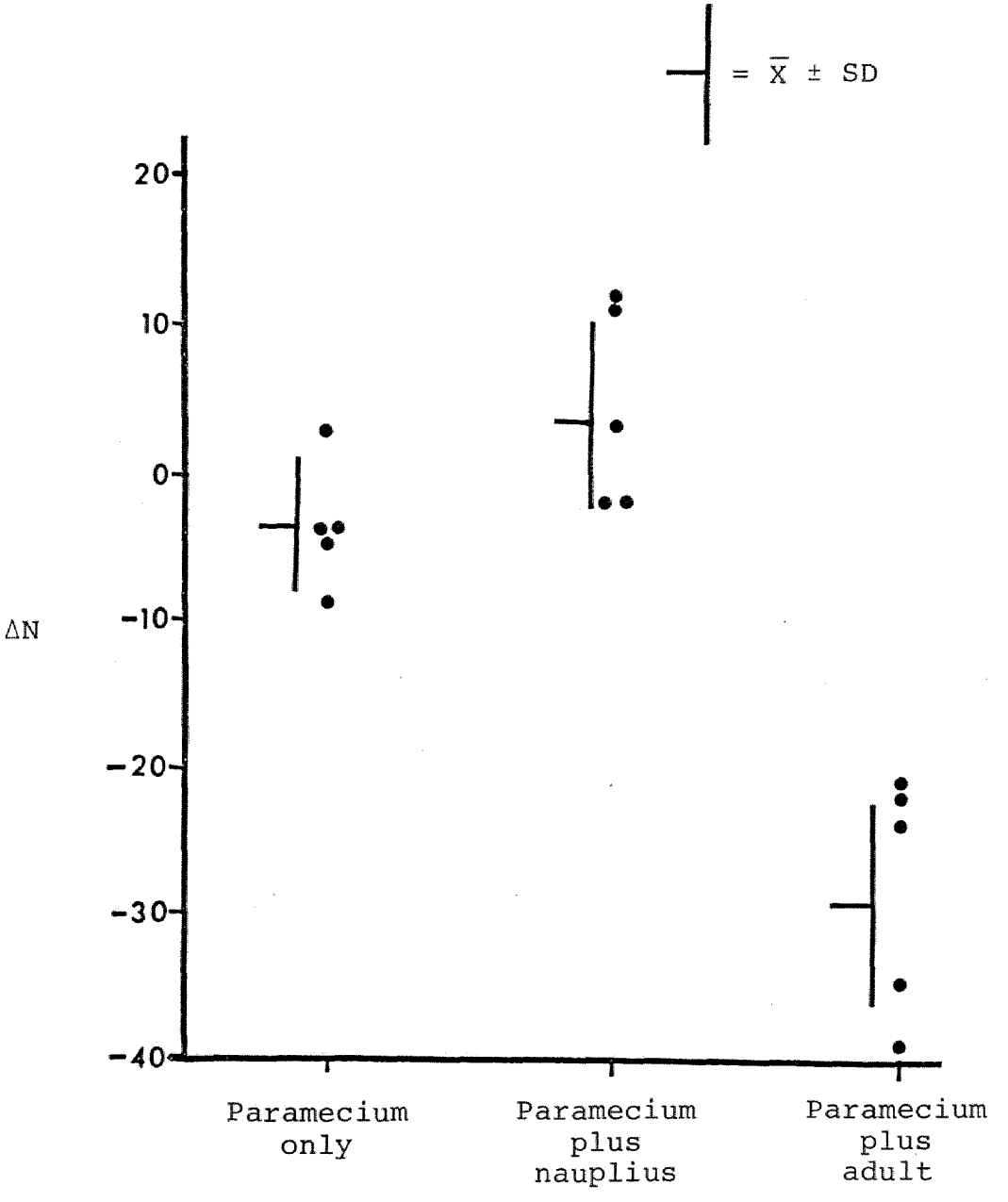

Fig. 3. Change in Paramecium numbers ($\Delta N = N_t - N_o$) due to three hours predation by C. b. thomasi. Horizontal bars represent means and vertical bars represent standard deviation.

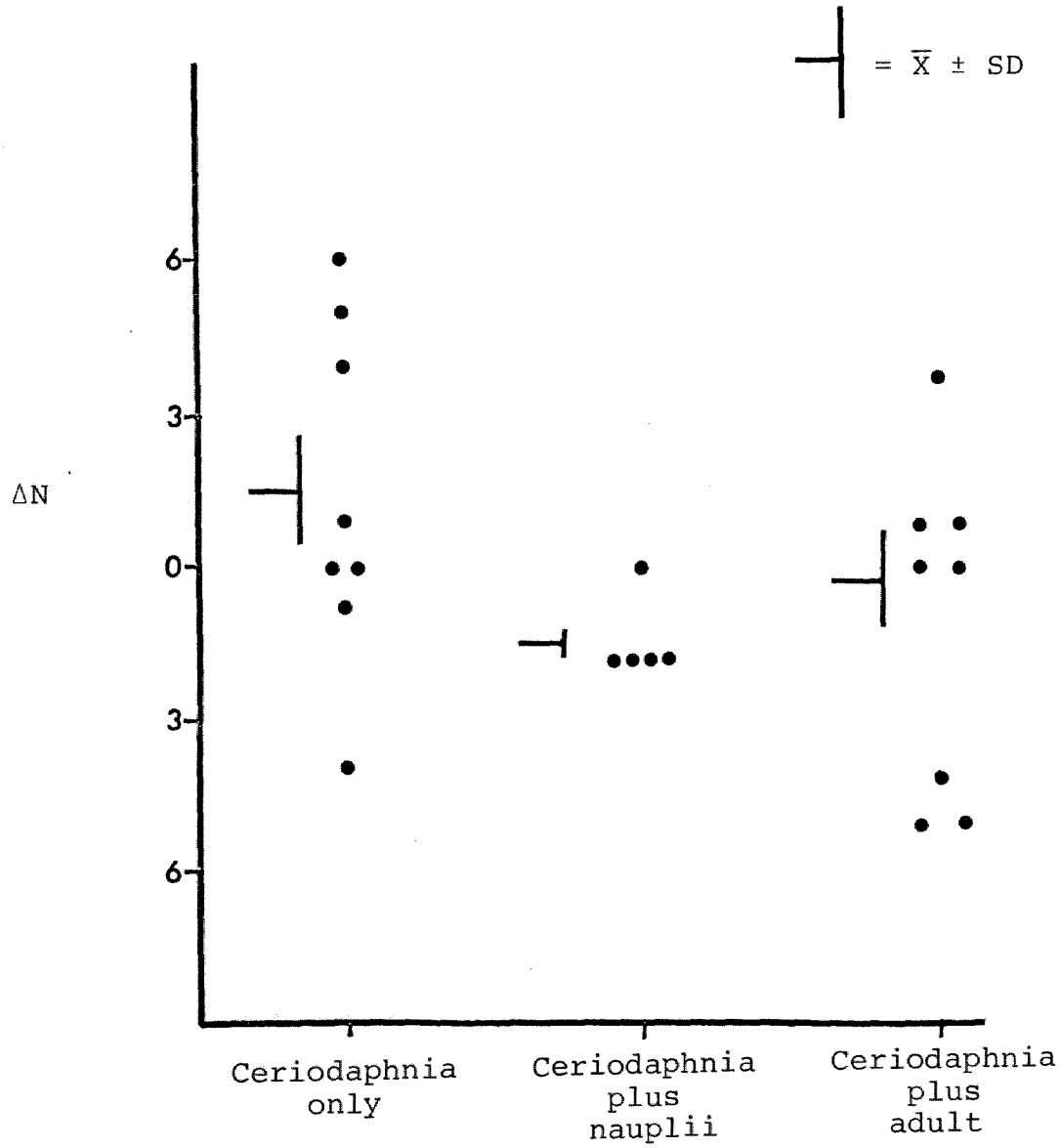

Fig. 4. Change in Ceriodaphnia numbers ($\Delta N = N_t - N_o$) after 27 hours contact with C. b. thomasi. Horizontal bars represent means and vertical bars represent standard deviation.

groups in contact with this predator. Naupliar C. b. thomasi did not predate on Paramecium, though they had an unexpected effect of stimulating an increase in numbers of this protozoan. The difference from controls was not significant, however, so this result is most likely an effect of random error. Adult C. b. thomasi have little effect on Ceriodaphnia (Figure 4). No significant difference exists between the adult experimental and control groups and the variability is similar in each. The effect of the nauplius is again peculiar. Mortality of Ceriodaphnia was higher in those vessels containing nauplii than in controls. Though the difference between experimentals and controls was not significant, the Mann-Whitney probability of such a departure was less than 0.1 and the variability was very small.

Predator gain method. No naupliar feeding was detected by the prey difference method. Consequently, other techniques suggested by the literature were attempted, the "predator gain" method to detect assimilation of radiotagged food and the "predator survivorship" method to investigate what diets can sustain individual cyclopoid adults and nauplii.

Table 4 lists corrected radioactive counts for fractions recovered from the pilot feeding trial. Most of the activity (1242 CPM/ml) in the food suspension is filtrate.

Table 4. Adult *Mesocyclops edax* fed ^{32}P-tagged *Aeromonas*: Counts per minute in various fractions.

1. Food suspension

specific activity = 1905 CPM/ml

bacteria fraction = 203 CPM/ml 14%

filtrate = 1242 CPM/ml 86%

$$\frac{203 + 1242}{1905} \times 100\% = 75.8\% \text{ recovery}$$

2. Recovered fractions after feeding trial for three groups.

	total activity introduced	predator	bacteria	filtrate	recovery
experimental	5715	128	441	3549	72%
control 1 (dead)	5715	134	800	3510	78%
control 2 (filtrate)	5715	121	378	7875	170%

Since bacteria were not washed and resuspended, the predators in the experimental group were exposed to both this filtrate and the bacterial cells during the feeding trial. Of the recovered fractions, the majority of counts are found in the filtrate and very little in the predators or bacteria. Most of the counts introduced were recovered (76%, 72%, and 78%) but there was a large positive error in the total counts recovered in the 2nd control (170%). The source of this error couldn't be determined. Graphic comparison between the counts for the predators from the experimental group and those from either control group showed no difference between groups. Thus, feeding was not demonstrated.

Survivorship Method. Survivorship studies were conducted during the summer of 1979, using adults and nauplii of C. vernalis and a variety of food types. Survival time (in days) was recorded for each group and compared to the group receiving no food (negative control). Significance of the survival time differences was established using the Mann-Whitney U-test. Two other parameters, molting by nauplii and nauplii production by adults, were also observed for each group.

Table 5 summarizes the experimental results for nauplii reared on no food, optimum food (mixed protozoan culture consisting of ciliates, green and colorless flagellates, yeast, bacteria, plant detritus, and dissolved

Table 5. Survivorship of naupliar C. vernalis under a variety of food conditions. Mean survival time (days) ± one standard deviation is indicated for each group. Mann-Whitney U-test values (U_s) and significance are computed by comparison of each group with the negative control (no food). Other symbols used are: "n" for the number of replicates and "d.e." for diatomaceaous earth (2-20 microns particle size). "Filtrate" refers to a 0.45 micron Millipore filtrate from a mixed protozoan culture.

food condition	n	survival time	U_s	signif. ($p \leq x$)	molt to copepodid?
no food	54	3.6±1.19	--	--	no
optimum food	45	17.9±9.60	2391	.005	yes
bacteria (Aeromonas)	54	3.8±1.30	1399	.35	no
bacteria plus d.e.	9	5.2±0.97	414	.005	no
algae (Selenastrum)	18	3.4±2.35	402	.52	no
filtrate (0 wash)	18	8.2±3.90	1006	.005	no
(1 wash)	18	4.9±1.74	895	.05	no
(2 washes)	18	5.7±1.57	997	.005	no
(3 washes)	18	5.1±1.53	960	.005	no
(4 washes)	18	5.7±1.65	997	.005	no

organics), bacteria (Aeromonas), bacteria plus diatomaceous earth, algae (Selenastrum), and 0.45 μ filtrate from the optimum food.

Survivorship of nauplii C. vernalis fed bacteria or algae, was not statistically different from survivorship in the negative control. All three groups starved in three to four days. When diatomaceous earth was added to act as an inert surface for bacterial attachment, nauplii reared survived significantly longer than those in the negative control group (5.2 vs. 3.6 days). Filtering a mixed protozoan culture through a 0.45 μ Millipore filter yielded a cell free solution, rich in dissolved organic substances, which was also tried as an experimental food source. Survivorship with this filtrate was greater than survivorship on all other experimental foods ($\bar{X}$ = 8.2 days). Because flagellates and bacteria were observed in the feeding chambers at the end of each day and these were suspected of being contaminants introduced with the nauplii, survivorship studies were done with nauplii which had been washed 1, 2, 3, and 4 times, and fed the above filtrate. Contaminants were not observed when nauplii were washed 2, 3, and 4 times. Survivorship in these groups was still significantly greater than the control.

Nauplii lived longest when fed the optimum diet, (positive control) surviving an average 17.9 days, or more than twice as long as the next best survival time. This

was also the only diet where individuals succeeded in molting to the 1st copepodid stage (14 of 45 individuals, taking an average of 22±3.8 days). All other diets were deficient, though nauplii did well on the filtrates.

Experiments which tested survivorship of adult C. vernalis are summarized in Table 6. Adult starvation time (no food) was considerably longer (12.1 days) than the corresponding time for nauplii (3.6 days). Adult cyclopoids fed bacteria lived a significantly longer life than those receiving no food, though the difference between groups is not large (14.4 vs. 12.1 days). Adults fed algae and those fed filtrate did not survive any longer than the control (11.4 and 10.8 days, respectively, vs. 12.1 days). Adults fed optimum food (protozoa culture) all lived to the end of the experimental period (35 days) and appeared alert and active at this time.

The optimum diet given the adults not only provided adequate nourishment for survival, but also energy to produce young. Cyclopoid adults fed the mixed protozoan culture produced an average of 6.2 batches of newborns per individual (maximum of 9 batches having 35-90 nauplii each), which compares well with the maximum number of egg clutches which can be produced from stored sperm. Pennak (1978) states that 7 to 13 batches of eggs can be produced from sperm of a single copulation. Since these experimental cyclopoids were kept isolated for the duration of this

Table 6. Survivorship of adult C. vernalis under a variety of food conditions. Mean survival time (days) ± one standard deviation is indicated for each group. Mann-Whitney U-test values (U_s) and significance are computed by comparison of each group with the negative control (no food). Other symbols used are: "n" for the number of replicates and "d.e." for diatomaceous earth (2-20 microns particle size). "Filtrate" refers to a 0.45 micron Millipore filtrate from a mixed protozoan culture.

food condition	n	survival time	U_s	signif. ($p \leq x$)	average number of newborn batches
no food	10	12.1±2.18	--	--	0.3
optimum food	5	35*	50	.005	6.2
bacteria (Aeromonas)	10	14.4±2.18	79.5	.05	0.3
algae (Selenastrum)	5	11.4±3.02	28.5	.50	0.4
filtrate (1 wash)	5	10.8±2.17	27.5	.50	0.2

*All individuals survived until the termination of the experiment at 35 days.

study, sperm would be a limiting factor for egg production. The latest date nauplii were produced in vessels receiving optimum food was the 23rd day, whereas none of the other groups produced nauplii past the 6th day.

Tables 7 and 8 summarize the feeding data (all three methods) from the current study for adult and naupliar cyclopoids, respectively. Adults were shown to feed on Paramecium, Asplanchna, and cyclopoid nauplii, but not on ostracods, Ceriodaphnia, Selenastrum, nor protozoan culture filtrate. The results for adult feeding on bacteria and Bosmina were ambiguous. Nauplii fed on protozoan culture filtrate and bacteria with diatomaceous earth, but not on bacteria alone, Selenastrum, and unknown alga, Paramecium, Asplanchna, Bosmina, nor Ceriodaphnia.

DISCUSSION

Three species of carnivorous cyclopoids have been used in the current study, Cyclops bicuspidatus thomasi, Mesocyclops edax, and Cyclops vernalis. These were the predominant cyclopoids during the summer of 1977, 1978, and 1979, respectively. Each species was not readily obtainable all three years. Since different experimental methods evolved during the course of the study, feeding trials occasionally used different species of predator. This, however, should not make much of a difference, since

Table 7. Summary of adult cyclopoid feeding from the three experimental methods used in the current study. If the difference between experimental and control was significant ($\alpha \leq .05$), then it was concluded that feeding did occur.

Predator	Prey	Method used	Did feeding occur?
Mesocyclops edax	bacteria	Pred gain (^{32}P)	no
Cyclops vernalis	bacteria	Survivorship	yes
Cyclops vernalis	algae (*Selenastrum*)	Survivorship	no
Cyclops vernalis	protozoan filtrate	Survivorship	no
Cyclops bicuspidatus thomasi	*Paramecium*	Prey difference	yes
Cyclops vernalis	*Asplanchna*	Prey difference	yes
Cyclops vernalis	*Bosmina* (12 hr. exp.)	Prey difference	no
Cyclops vernalis	*Bosmina* (48 hr. exp.)	Prey difference	yes
Cyclops bicuspidatus thomasi	cyclopoid nauplii	Prey difference	yes
Cyclops bicuspidatus thomasi	ostracods	Prey difference	no
Cyclops bicuspidatus thomasi	*Ceriodaphnia*	Prey difference	no

Table 8. Summary of naupliar cyclopoid feeding from three experimental methods used in this study. All nauplii are of the 1st instar (NI). "d.e." refers to diatomaceous earth, particle size 2-20 μ.

Predator	Food	Method used	Did feeding occur?
Cyclops bicuspidatus thomasi	bacteria	Prey difference	no
Cyclops vernalis	bacteria	Survivorship	no
Cyclops vernalis	bacteria plus d.e.	Survivorship	yes
Cyclops bicuspidatus thomasi	unknown algae	Prey difference	no
Cyclops vernalis	algae (*Selenastrum*)	Survivorship	no
Cyclops vernalis	protozoan filtrate	Survivorship	yes
Cyclops vernalis	prot.filt. (pred. washed)	Survivorship	yes
Cyclops bicuspidatus thomasi	*Paramecium*	Prey difference	no
Cyclops vernalis	*Asplanchna*	Prey difference	no
Cyclops vernalis	*Bosmina* (12 hr. exp.)	Prey difference	no
Cyclops vernalis	*Bosmina* (48 hr. exp.)	Prey difference	no
Cyclops bicuspidatus thomasi	*Ceriodaphnia*	Prey difference	no

the three species have similar feeding styles and habits.

Fryer (1957b) studied the feeding habits of a variety of cyclopoids by examining their gut contents. He found that some were wholely herbivorous, while others were carnivorous. The carnivorous species were able to feed on a wide variety of animals (other crustacea, diptera larva, and oligochaetes) but not on algae. One exception, M. leuckarti was also found to ingest diatoms along with animal prey. Undoubtedly, soft-bodied prey were taken also, but were not in a recognizable form, so they were not listed. Fryer concluded that all of the carnivorous species feed on similar prey. C. b. thomasi, M. edax, and C. vernalis are all known to be carnivorous (Anderson, 1970; Brandl and Fernando, 1978; Confer, 1971; Dodson, 1975; Fryer, 1957b; McQueen, 1969) and all three species have been shown to be cannibalistic (Brandl and Fernando, 1978; Loundes, 1928, in Fryer, 1957b; McQueen, 1969). Anderson (1970) found that C. b. thomasi and C. vernalis both ate similar prey, with each consuming naupliar and copepodid Diaptomus and the cladoceran Diaphanosoma. Their rates of feeding differed considerably, however (highest feeding rate = 0.27 and 0.014 prey eaten/predator per hour for C. vernalis and C. b. thomasi, respectively).

Therefore, the three species of cyclopoids considered in the current study, adult stages of C. b. thomasi,

M. edax, and C. vernalis, can be pooled together as "carnivorous cyclopoids" when considering qualitative feeding. Individual species must be considered separately when discussing adult quantitive feeding. No conclusions can be made for naupliar cyclopoids, however, since less is known about their feeding abilities.

Adult feeding. The results of the current feeding studies using adult cyclopoid copepods compares favorably with known carnivores previously described (Table 9). In the literature, Cyclops vernalis and Cyclops viridis are known under the subgenus name, Acanthocyclops (Yeatman, 1959).

Mesocyclops edax did not feed on ^{32}P tagged bacteria (Aeromonas hydrophilia) at a density of 1×10^8 cells/ml. Cyclops vernalis, tested under a variety of food conditions in survivorship studies (Figures 5 and 6), were nourished slightly by the same bacteria at a density of 10^6 to 10^7 cells/ml. Though there may be some species specific differences in feeding ability on this resource, methodology most likely explains the differences in results. Tagged bacteria in the current study had a low specific activity (2CPM/10^6 bacteria); this would require each predator ingest a very large number of cells to be readily detected. Apparently they did not, for no significant differences existed between experimentals and controls. In the survivorship study, adult C. vernalis survived only an

Table 9. Comparison of qualitative feeding data from current study and representative data from other researchers. All of the predators are species of carnivorous cyclopoid copepods, stages CIV, CV, and/or adults.

Predator	Prey	Predation?	Researchers	Method Used
M. edax	bacteria	no	CURRENT STUDY	Predator gain (^{32}P)
C. viridis	bacteria	no	Monakov and Sorokin (1960)	Predator gain (^{14}C)
M. leuckarti	bacteria	no	Monakov and Sorokin (1960)	Predator gain (^{14}C)
C. vernalis	algae	no	CURRENT STUDY	Predator survivorship
C. vernalis	algae	no	Fryer (1957b)	Gut contents
M. leuckarti	algae	yes	Fryer (1957b)	Gut contents
C. b. thomasi	*Paramecium*	yes	CURRENT STUDY	Prey difference
C. b. thomasi	*Paramecium*	yes	CURRENT STUDY	Direct observation
C. b. thomasi	*Paramecium*	yes	Martin and Novotny (1975)	Predator survivorship
C. vernalis	*Paramecium*	yes	CURRENT STUDY	Predator survivorship
C. vernalis	*Asplanchna*	yes	CURRENT STUDY	Prey difference
C. vernalis	*Asplanchna*	yes	Li and Li (1979)	Direct observation

Table 9 (continued)

Predator	Prey	Predation?	Researchers	Method Used
C. vernalis	*Bosmina*	yes	CURRENT STUDY	Prey difference
C. vernalis	*Bosmina*	yes	Kerfoot (1978)	Direct observation
C. b. thomasi	*Ceriodaphnia* adults	no	CURRENT STUDY	Prey difference
C. vernalis	*Ceriodaphnia* adults	no	Li and Li (1979)	Direct observation
C. b. thomasi	*Ceriodaphnia* adults	yes	Anderson (1970)	Prey difference
C. b. thomasi	*Ceriodaphnia* adults	no	McQueen (1969)	Prey difference
C. b. thomasi	*C. b. thomasi* nauplii	yes	CURRENT STUDY	Prey difference
C. b. thomasi	*C. b. thomasi* nauplii	yes	McQueen (1969)	Prey difference
C. b. thomasi	*C. b. thomasi* nauplii	no	Anderson (1970)	Prey difference
C. b. thomasi	*C. b. thomasi* nauplii	yes	Martin and Novotny (1975)	Direct observation

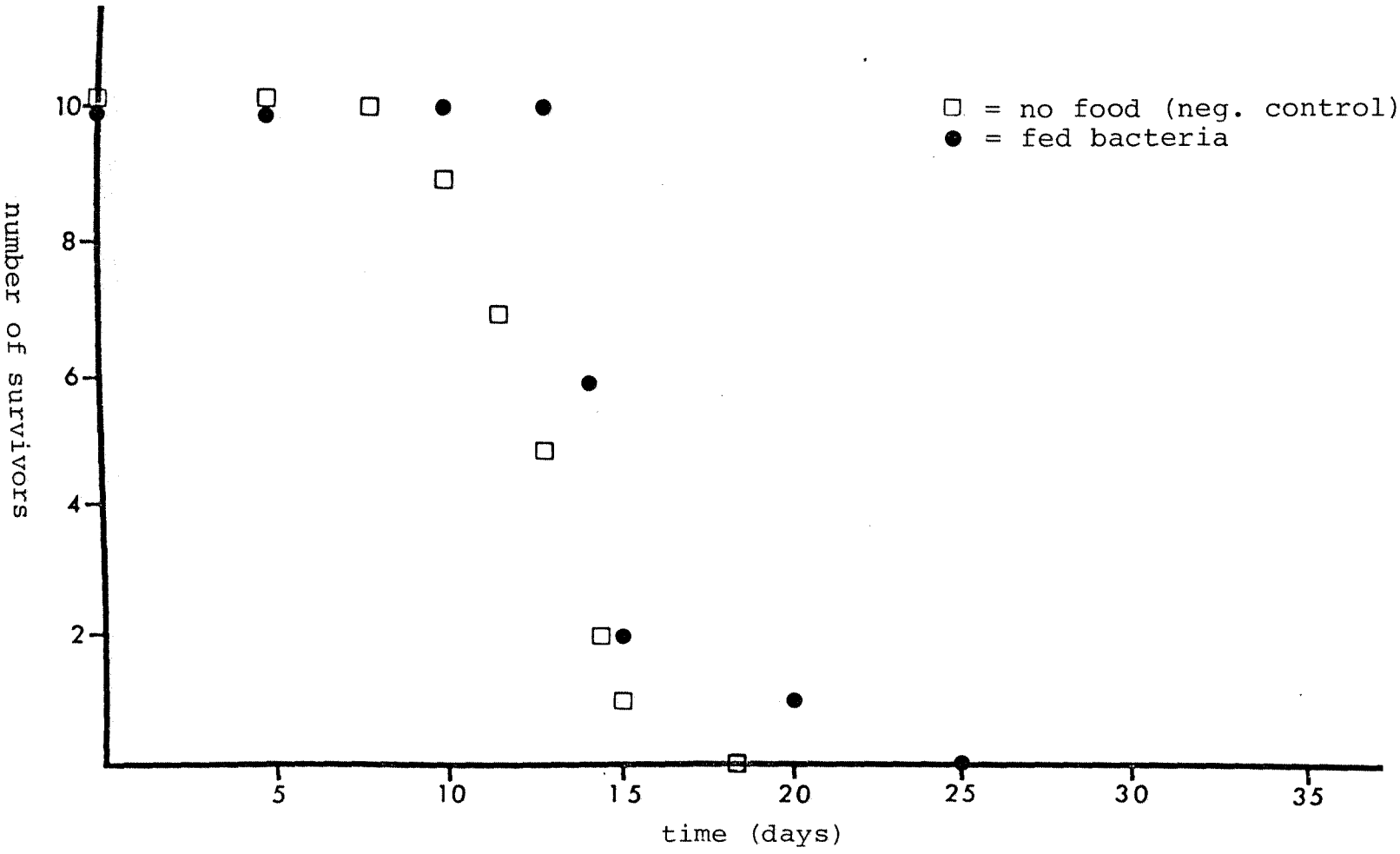

Fig. 5. Survivorship of adult C. vernalis fed bacteria and no food.

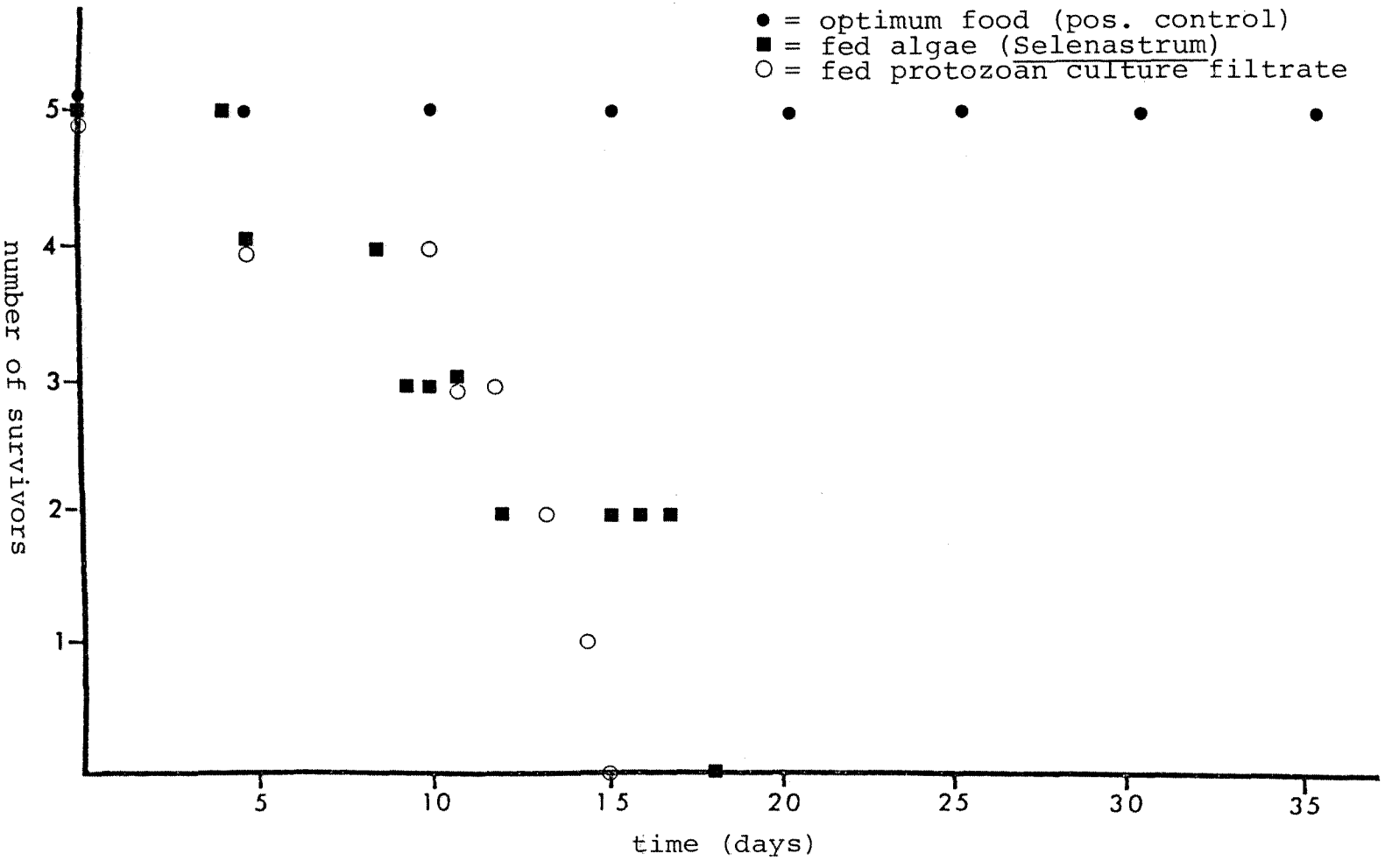

Fig. 6. Survivorship of adult C. vernalis fed optimum food, algae, and protozoan culture filtrate.

average 2.3 days longer on bacteria than starvation controls. Though this difference was significant ($p<.05$), it does not indicate long term survival. Calculations (Appendix, Table 11) show that if a mature cyclopoid was fed a diet only of bacteria, it would require assimilation of 10^7 cells/day. If the animals had assimilated this amount, they could have been detectable by the radioisotope method.

The bacterial densities used in the current study are representative of that occurring in natural systems. Freshwater concentrations may range from 10^6 cells/ml in the pelagic zone (Gak, 1972) to 10^{10} cells/g-detritus in the benthos (Fenchel and Harrison, 1976).

Monakov and Sorokin (1960), using the predator gain technique (^{14}C), found that adult Cyclops viridis and Mesocyclops leuckarti were unable to feed on bacteria (mixed heterotrophs) at a concentration of 2×10^6 cells/ml. At much higher densities (1×10^{10} cells/ml), C. viridis assimilated a small amount, while M. leuckarti still did not. They suggest that C. viridis may be able to utilize bacteria-detritus complexes. This species is commonly found in littoral and benthic regions, whereas M. leuckarti is wholely pelagic in habit. The reason Monakov and Sorokin were able to detect uptake of tracer in the predator may have been because of higher specific activity of the bacteria. The authors did not test survivorship of their

animals on bacteria. Therefore, from the data of the current study and that of Monakov and Sorokin (1960), I conclude that bacteria alone do not provide adequate food for the adult carnivorous cyclopoid.

Cyclops vernalis adults fed green algae (Selenastrum) survived no longer than the starvation controls. This result supports Fryer's (1957b) observation of the gut contents of this species. C. vernalis which Fryer collected from the field contained oligochaetes, cyclopoid copepodids, chydorid cladocerans, and rotifers, but no algae, as did most other carnivorous cyclopoids he examined. I conclude, most adult carnivorous cyclopoids do not feed on algae.

Feeding trials and direct observations revealed that C. b. thomasi adults predate on Paramecium. During the survivorship studies, C. vernalis adults thrived on a diet of mixed protozoa. Martin and Novotny (1975) obtained similar results in rearing pure cultures of C. b. thomasi, and Coker (1933, in Fryer, 1957b) reared C. vernalis on mixed protozoan culture. Clearly, large ciliates are easily captured by these cyclopoid adults. I conclude that carnivorous cyclopoids capture and ingest ciliated protozoa and probably also ingest other components of mixed protozoan cultures, insuring long term survival and fecundity.

Rotifers are commonly found occurring with the copepods in freshwater habitats (Wetzel, 1975). Cyclops vernalis caused significant losses in populations of

Asplanchna during prey difference trials. Li and Li (1979) observed attacks by C. vernalis on Asplanchna and, in fact, found that this prey type was selected most frequently over all other prey offered (Bosmina, Ceriodaphnia, Daphnia, Diaphanosoma, and Diaptomus) in their multi-prey experiments. They supported their results by observing prey characteristics such as body shape, swimming speed, and predator avoidance behavior. These observations showed that Asplanchna could be caught by this cyclopoid. Therefore, I conclude that carnivorous cyclopoids readily capture large rotifers and, when numerous, exert a major influence on their populations.

The two prey difference experiments of C. vernalis adults on Bosmina, 48 hours and 12 hours duration, gave mixed results with the former experiment indicating significant predation and the latter experiment indicating no predation. There are two possible reasons for this discrepancy. First, the longer trial length of the first experiment allowed more chances for contact between predators and their prey, resulting in a higher number of successful captures. A second possibility is that since the Bosmina were not fed during the feeding trials, those in the 48 hour trial became weak from lack of food and less able to evade predators. Li and Li (1979) reported that search and capture of Bosmina by C. vernalis was difficult and time consuming. At least one of their predators required

48 hours to capture a Bosmina. Kerfoot (1978) observed cyclopoid (Cyclops bicuspidatus and Cyclops vernalis) predation on Bosmina using high speed photography. Though the cyclopoids successfully fed on these small-bodied cladocera, the prey's body shape and swimming movements made them difficult to capture, necessitating repeat attacks. Bosmina's predator avoidance behavior, called the "dead man's response" (Kerfoot, 1978) often foils the predator by reducing vibration cues.

A major difference exists between my Bosmina results and those of Li and Li (1979). They reported that after predation, the carapace usually remained behind. In my feeding trials of the current study (48 hours, Table 2), most of those animals predated were gone from the dishes entirely. This is seen by comparing the number unaccounted (initial minus final counts) in experimental and control vessels. A much larger number of individuals disappeared from the adult experimental vessels (mean difference = 15.4) than from either the controls (0.8) or the nauplius experimentals (-1.8). This difference indicates that adults were clearly removing Bosmina. The reason for the discrepancy between the current study and Li and Li's study lies in experimental procedure. The 48 hour feeding trials were set up and left unattended until their conclusion. At that time, remains of dead animals were removed and recognizable forms were counted. The remaining live animals

were killed and counted. Li and Li used smaller vessels containing only a few prey and made observations every four hours, terminating their experiments as soon as the prey had been killed. Since cyclopoids often search along the bottom of laboratory glassware, with longer contact time it would not be surprising if the cyclopoids completely removed the Bosmina. From these studies, I conclude that carnivorous cyclopoids predate on Bosmina and with time the prey are removed.

In the current study, C. b. thomasi did not predate on Ceriodaphnia adults during prey difference trials, though unsuccessful attacks were observed. Li and Li (1979) obtained similar results using the predator, C. vernalis. They found Ceriodaphnia adults to be one of the least preferred prey in their multi-choice experiments, and observed that these large cladocera were only captured when at very high densities when the head was vulnerable to attack. The reason for Ceriodaphnia's success at surviving contact with this predator is anatomical rather than behavioral. Ceriodaphnia is apparently too large for capturing, although their escape movements attract cyclopoid attacks. In contrast to the adults, Ceriodaphnia neonates were readily captured and consumed. Brandl and Fernando (1975) found M. edax to also consume Ceriodaphnia neonates. Cyclopoid predation still has its impact on the population.

Anderson (1970) found in a "three-prey-choice"

experiment, C. b. thomasi stages CIV and CV chose Ceriodaphnia 75% of the time, Diaptomus copepodids 25%, and C. b. thomasi NV-NVI 0%. Data from other studies (Li and Li, 1979; McQueen, 1969; current study) would indicate that the order of choice should be the reverse. Anderson does not indicate which stage of Ceriodaphnia he used, though one would expect he used adults. If neonates were used, then his results would not be unusual. The species he used (C. quadrangula) is no smaller than the Ceriodaphnia used by Li and Li (1979), so species differences are probably not the explanation for the difference. Though the discrepancy between Anderson's results and those other researchers remains unresolved, I conclude that carnivorous cyclopoids rarely are successful at capturing Ceriodaphnia adults.

The prey difference experiment testing cyclopoid feeding on ostracods (Table 2) is not considered in Table 9 because nowhere else has this feeding interaction been studied. Ostracods are commonly found in the benthos of a wide variety of freshwater and marine environments (Meglitsch, 1972). Because some ostracods are occasionally found swimming above the substrate (Pennak, 1978) and some species of cyclopoids invade the benthos (Monakov and Sorokin, 1960), members of the two groups probably come in contact with one another. In the current study, prey difference trials showed no predation by C. b. thomasi adults on populations of ostracods. Considering the anatomy and

behavior of the ostracod, the absence of feeding isn't surprising. The ostracod's head and trunk are completely enclosed in a carapace and its body is thick. Consequently, vital parts are protected and the body as a whole is probably difficult to grasp. When the ostracod is disturbed, it draws its appendages inside the carapace and stops moving. This may further serve as protection by not producing predator-exciting vibrations. I conclude that carnivorous cyclopoids do not predate on ostracods, though the two occasionally co-exist.

Cannibalism by C. b. thomasi adults was demonstrated in prey difference trials. The adults had a large impact on the newborn nauplii, removing an average 12.6 (of 20) nauplii during the 20 hour trial. The density of predators and prey used were 50 and 1000/1, respectively, 5X and 20X more dense than the concentrations of M. edax adults and mixed nauplii reported in local field samples by Welchlin (1975). The results from the current study agree with McQueen's (1969) and Martin and Novotny's (1975) studies of C. b. thomasi, Brandl and Fernando's (1978) study of M. edax, and Loundes' (1928, in Fryer, 1957b) and Robertson et al's (1974) work with C. vernalis, but not with Anderson's (1970) work with C. b. thomasi and C. vernalis.

McQueen (1969) found that adults and copepodids CIV and CV of C. b. thomasi removed a large proportion of C. b. thomasi naupliar standing stock (NI-NV stages). Anderson

(1970), however, saw no evidence of cannibalism by C. b. thomasi nor C. vernalis in his prey difference trials. This could be because he used only instars NV, NVI, and CI of the prey. I used only the 1st naupliar instar. In his study of arctic ponds, Dodson (1975) found that the dominant invertebrate predators, C. vernalis and Heterocope septentrionalis, cannibalized at very low rates but took each others larvae at high rates.

Thus, although there is some difference of opinion, it appears that those species of cyclopoid nauplii examined in the current study suffer mortality from mature cyclopoids. Gehrs and Robertson (1975) showed that the highest mortality rates occurred during the egg to fourth naupliar interval in a natural population of Diaptomus. If the same situation exists for cyclopoid nauplii, then it would appear that during periods of high adult cyclopoid populations, the greatest population regulator for cyclopoids would come from within the population.

McQueen's in-lab feeding rate for his (1969) predator prey system was 2.0 prey per predator per day, considerably lower than the 15.1 nauplii per C. b. thomasi per day in the current study. The prey density in McQueen's study was 300/1, while mine was 1000/1 and copepod predators are known to follow Holling's (1966) functional response to prey density. A second and equally important reason for the difference is that I used only nauplii of

stage NI while McQueen used a mixture of NI-NV. The later instars, being larger, are higher in caloric value.

The current study and McQueen's (1969) study may overestimate the effects of cannibalism on copepod populations, since the predators were starved 12 to 24 hours prior to the feeding experiments. Friedman and Strickler (1975) suggest that the chemoreceptors found on the mouthparts of copepods may be useful to detect members of their own species, thus inhibiting cannibalistic behavior in natural unstarved populations. If their hypothesis is correct, then the current study suggests that nauplii develop chemical cues which signal "hands off" to potential adult cannibals. An alternate explanation is that superior swimming and sensory abilities allow the later stage nauplii to avoid these predators. This latter hypothesis could be tested by methods such as Strickler's (1975a, 1975b). Since copepods reproduce slowly in comparison to other zooplankters (Porter, 1975) and there is little overlap between the feeding of cyclopoid adults and their nauplii (current study), there should be little benefit to cannibalism in natural cyclopoid populations.

I conclude that carnivorous cyclopoids predate on their own offspring, especially the early (NI-NIV) stages, and at high cyclopoid densities this predation would have a major limiting effect on these populations.

Naupliar feeding. The results of the current feeding

studies using naupliar cyclopoids are different from previously described studies (Table 10). Since naupliar feeding on large prey (Paramecium, Asplanchna, Bosmina, and Ceriodaphnia) is not considered elsewhere in the literature, these results are not included in Table 10.

In prey difference trials, C. b. thomasi nauplii did not predate on Paramecium nor Ceriodaphnia and C. vernalis nauplii did not predate on Asplanchna nor Bosmina. This is not surprising, for each is greater than or equal to the size of the nauplius (Table 1) and each is motile. Newborn nauplii are responsive to the touch of a probe and to water currents created with a pipette. These are apparently instinctive anti-predator responses. Direct observations supported this hypothesis that nauplii avoid Paramecium, Asplanchna, Bosmina, and Ceriodaphnia a priori. I conclude that newborn nauplii do not predate on large motile animals such as Paramecium, Asplanchna, Bosmina, and Ceriodaphnia.

C. b. thomasi nauplii did not feed on bacteria (E. cloacae, final viable density = 800 - 1000 cells/ml, when tested using the prey difference method. Though there was not a significant difference between final counts of experimental and control vessels, the data suggest that presence of the nauplius promotes an increase in bacterial numbers. Such an increase could be caused by (1) naupliar excretion products (2) bacteria in nauplius feces (3) bacterial contaminants on the nauplius body surface. Because of the

Table 10. Comparison of naupliar feeding data from the current study and representative data from the literature. Only food types used both in the current study and by others are considered in this table. Nauplii are from a variety of copepod species, some in which the adult stage's feeding habits (A) are herbivorous (H), others carnivorous (C). Abbreviations for predator species are: Cbt-*Cyclops bicuspidatus thomasi*, Cve-*Cyclops vernalis*, Cvi-*Cyclops viridis*, Ml-*Mesocyclops leuckarti*, Cf-*Calanus finmarchicus*, and Ej-*Euchaeta japonica*.

Pred	(A)	Instar	Food	Feeding?	Researchers	Method used
Cbt	(C)	NI	bacteria	no	CURRENT STUDY	Prey difference
Cve	(C)	NI	bacteria	no	CURRENT STUDY	Survivorship
Cvi	(C)	?	bacteria	yes	Monakov and Sorokin (1960)	Predator gain (^{14}C)
Ml	(C)	?	bacteria	yes	Monakov and Sorokin (1960)	Predator gain (^{14}C)
Cve	(C)	NI	bacteria plus diatomaceous earth	yes	CURRENT STUDY	Survivorship
Cbt	(C)	NI	unknown algae	no	CURRENT STUDY	Prey difference
Cve	(C)	NI	algae (*Selenastrum*)	no	CURRENT STUDY	Survivorship
Cf	(H)	NI	algae (*Prorocentrum*)	no	Marshall and Orr (1956)	Predator gain (^{32}P)
Cf	(H)	NIII	algae (*Prorocentrum*)	yes	Marshall and Orr (1956)	Predator gain (^{32}P)
Cvi	(C)	NI	algae (*Scenedesmus*)	yes	Smyly (1970)	Survivorship
Cve	(C)	NI	protozoan filtrate	yes	CURRENT STUDY	Survivorship
Ej	(H)	NIII	organic solutes	yes	Lewis (1967)	Survivorship

increase in bacterial numbers, naupliar feeding, if it did occur, is masked. Therefore, data from this experiment is not definitive.

According to the naupliar survivorship studies (Figures 7 and 8), C. vernalis did not assimilate bacteria in particle-free suspensions, for they starved to death in the same length of time (3-4 days) as those in the negative control group. It is unlikely that the nauplii were poisoned by the concentration of bacteria to which they were exposed (10^6-10^7 cells/ml), for this concentration is no higher than that which the nauplius would encounter in its natural environment (Gak, 1972) and Aeromonas is commonly found in freshwaters (Breed et al., 1957).

The results from the current study do not agree with Monakov and Sorokin (1960), who determined, using the predator gain method (^{14}C), that Cyclops viridis nauplii and Mesocyclops leuckarti nauplii ingest and assimilate bacteria at naturally occurring concentrations. They calculated that the quantity assimilated would be high enough for normal growth and development. There are several possible reasons for this difference.

First, there may be differences in mouthpart structure between the different species used. This seems unlikely, however, since C. vernalis and C. viridis are closely related (subgenus Acanthocyclops). A second reason for the difference may lie in the composition of the feeding

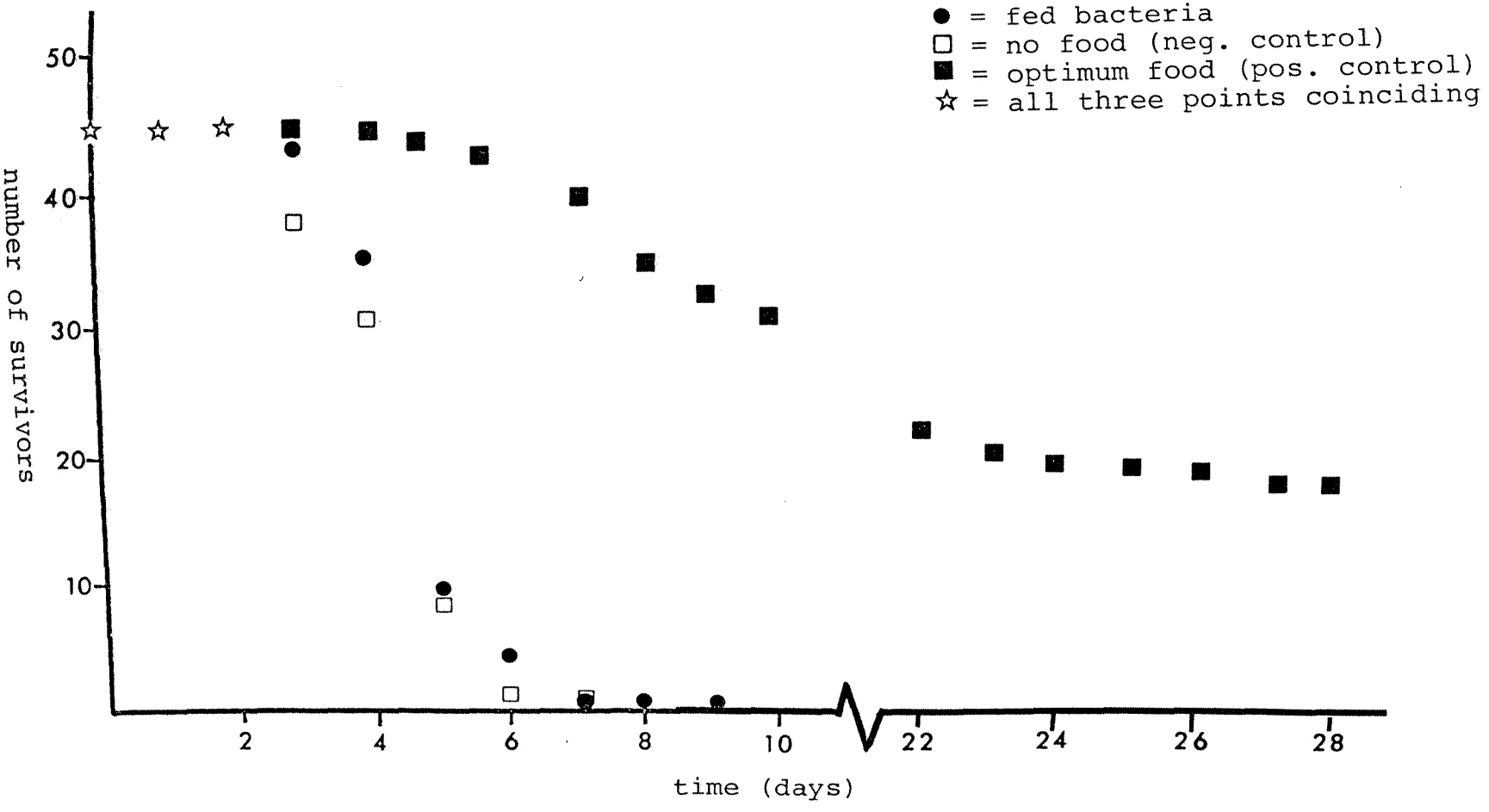

Fig. 7. Survivorship of C. vernalis nauplii fed bacteria, no food, and optimum food.

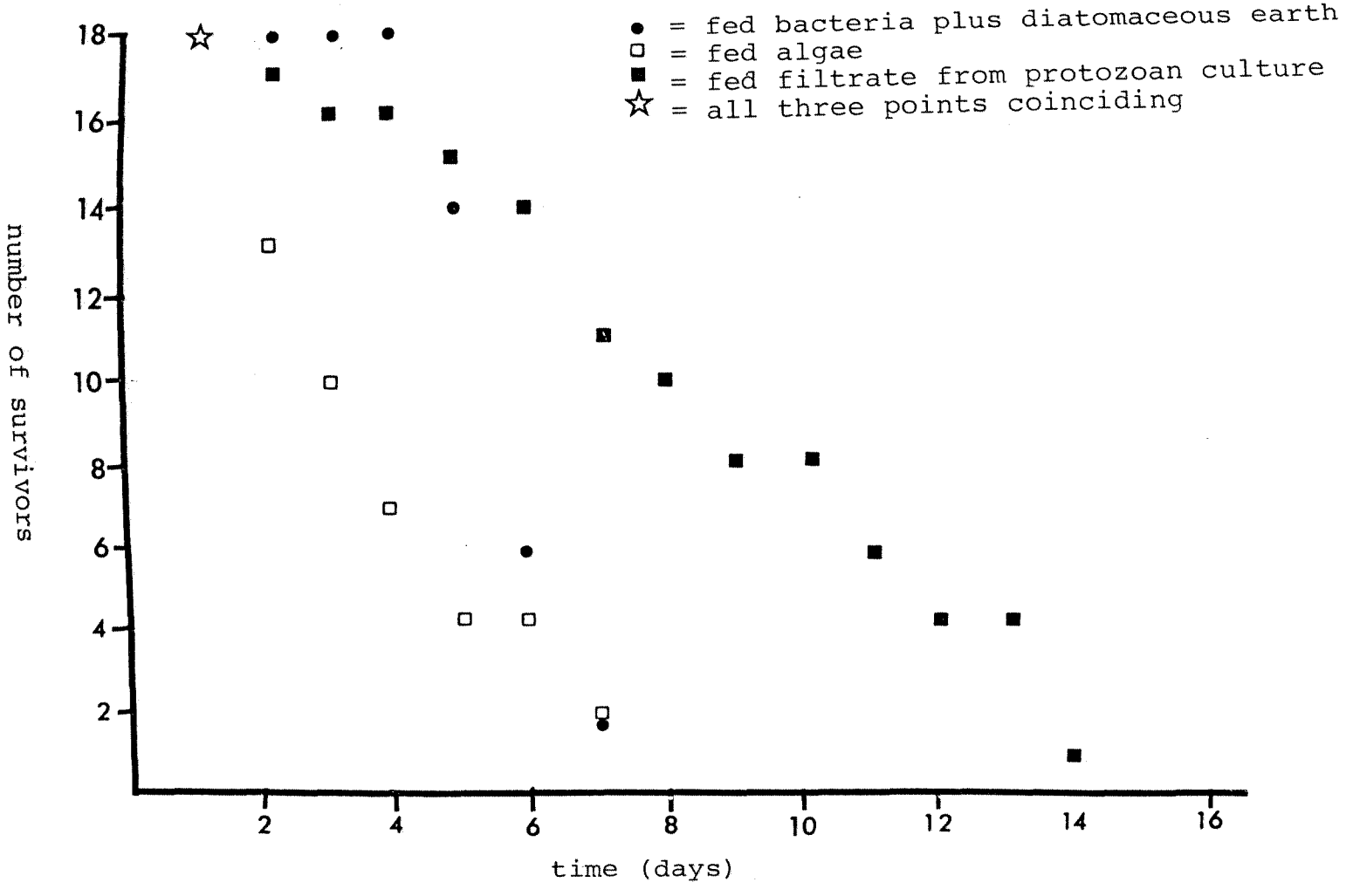

Fig. 8. Survivorship of C. vernalis nauplii fed bacteria plus diatomaceous earth, algae, and protozoan culture filtrate.

solution. While I used a pure culture of Aeromonas and sterile, particle-free solutions, Monakov and Sorokin used mixed heterotrophs and did not define its purity. If large particles had been present, these could have been used for bacterial attachment. Setules found on the antennal and mandibular spines of Calanus nauplii are about 3 μ apart (Marshall and Orr, 1956) which would allow bacteria the size of Aeromonas to slip through. So bacteria coated particles could be captured by the nauplius. In the current study, nauplii fed on bacteria adsorbed to diatomaceous earth (2-20 μ) but not on bacteria alone. Feeding of bacteria-coated particles is not unusual. Bacteria is often associated with particles in freshwaters (Baker and Bradnam, 1976) and serve as nutrition for benthic invertebrates, such as dipteran larvae (Baker and Bradnam, 1976) amphipods (Marzolf, 1966), oligochaetes (Brinkhurst and Chua, 1969), and snails (Calow, 1974). Peterson et al. (1978) report that Daphnia pulex, D. middendorffiana, and D. longiremis filter bacteria, though not as efficiently as they filter yeast cells.

Difference in methods provides another explanation for differences in our results. The current food solution used was a pure culture, and such cultures are not success ful for long term survivorship (Provasoli et al., 1959).

I conclude that cyclopoid nauplii do not feed on bacteria in a particle free solution, do feed on bacteria

when particles (2-20 μ) are present, but survive poorly in either case when other nutrients are not present.

Feeding experiments with algae provided negative results. Prey difference trials testing C. b. thomasi nauplii on an unknown alga showed that the nauplii didn't feed (Table 2) and testing C. vernalis nauplii showed very poor survivorship on Selenastrum. These results contrast with the popular opinion that most nauplii are important grazers of phytoplankton.

Paffenhöfer (1971) reared Calanus helgolandicus from NIV to the adult stage on a diatom-dinoflagellate mixture and Smyly (1970) reared Cyclops viridis nauplii from hatching to adult on mixed species of algae. Both were mixed, non-axenic cultures. Survivorship studies which I conducted, though using cells of a correct size, shape (small aggregates, no spines), and behavior (non-motile) (Gauld, 1959; Porter, 1975), used a monospecific culture. The survivorship was very poor, indicating the nauplii were deriving no nutritional benefit. Provasoli (Marshall, 1973) demonstrated that copepods survive very poorly on unispecific cultures of protozoa or algae, requiring nutrients from several sources.

C. b. thomasi NI would not feed on the unidentified alga in prey difference trials. Two reasons may account for this. First, some nauplii do not feed until later stages. For instance, Marshall and Orr (1956) found that Calanus

helgolandicus did not feed on an alga during the NI stage, but did during and after the NIII stage. C. b. thomasi may have similar behavior.

The second reason for lack of feeding is that this particular alga may have been a poor choice for food. It was the most abundant member of the phytoplankton of the eutrophic Waterworks ponds (Des Moines) during a mid-summer plankton bloom, which suggests it was a blue green alga, some members of which are toxic to zooplankton (Porter, 1975). It is also noteworthy that few cyclopoid adults or nauplii were found existing in the same pond during this period. High counting errors make this particular experiment difficult to clarify.

I can come to no clear conclusion regarding the feeding of cyclopoid nauplii on algae. Prey difference results were unclear and survivorship studies showed that this food alone was not adequate.

Naupliar survivorship studies revealed that a mixed protozoan culture (also called "optimum food") served as a suitable nutriment for long term survival and development (Figures 7 and 8, Table 5). This diet served as a positive control, demonstrating that nutrition and not experimental manipulation was the source of most mortality. Since this culture contained a wide variety of components (Paramecium, mixed flagellates, yeast, bacteria, plant detritus, dissolved organics), the nauplii could have been consuming a

number of different fractions. Nauplii were shown to feed on dissolved organics (the filtrate) and to feed slightly on a bacteria/diatomaceous earth suspension, so in the mixed protozoan culture nauplii were feeding on dissolved organics and bacteria-coated particles. Neither dissolved organics nor bacteria plus diatomaceous earth provided as high a survivorship as the mixed protozoan culture, so other components may have been used. It is unknown whether or not nauplii can feed on small flagellates or yeast, though the data of Table 5 suggest that flagellates were utilized. Some flagellates and bacteria were observed at the end of each day in vessels receiving protozoan culture filtrate. Those nauplii washed still survived significantly longer than the control, demonstrating that the dissolved organics were used as a food resource. But washed nauplii also survived a significantly shorter time than the unwashed, so apparently the flagellates developing in the feeding chambers did provide some nutrition.

Negative controls died in 3-4 days. Dagg (1977), working with laboratory populations of Acartia and Pseudocalanus, found their starvation time to be only about 2 days, but other genera lived slightly longer. Because the natural environment is patchy with respect to food resources, nauplii may suffer rapid mortality from starvation. Under such circumstances cannibalism by the adult wouldn't be very detrimental to the population.

Naupliar survivorship on protozoan culture filtrate (0.45 μ) was longest of any experimental food with an average survival time of 8.2 days for unwashed nauplii and 5.4 for washed nauplii (1, 2, 3, 4 washes pooled). This food did not allow any individuals to survive until the copepodid stage.

Little information exists in the literature regarding copepods feeding on dissolved organic matter (DOM). Putter's (1913, in Esterly, 1916) calculations showed zooplankton food requirements higher than could be supported by ingesting particulates, indicating DOM might be used. Euchaeta japonica, grown in filtered seawater enriched with vitamins, had better survivorship from the egg to feeding (N3) stages than those grown without vitamins (Lewis, 1967). Vitamins are part of a rapidly utilized pool of DOM; consequently, their concentrations are often low (Wetzel, 1975) and limiting. Additions of nutrient solutions should stimulate growth, especially if other foods are not present. Lewis added inorganic salts plus vitamins; I added a filtrate rich in vitamins and amino acids. Two components in the protozoan culture, Cerophyl and yeast, are rich in plant tissue, nucleic acids, amino acids, and vitamins.

Of what use to the nauplius are these dissolved organic substances? First, they may serve as a direct energy source to the animal. Uptake could occur by swallowing water and absorbing nutrients through the gut. DOM

is the largest compartment of the carbon pool in freshwaters (Wetzel, 1975). Consequently, its use is very important to the energy budget of ecosystems. Second, these organics are quickly taken up by bacterial populations and a number of bacterial cell types are found associated with copepods. These bacteria may provide useful substances for the copepod.

Implications of the current study. Carnivorous cyclopoid adults and their nauplii have considerably different food habits, so they do not compete for food resources. Adults specialize in feeding on other zooplankters, while nauplii utilize bacteria-coated detritus, dissolved organics, and possibly flagellates. Since adult cyclopoids are limited to prey of specified size ranges, the impact of adult predation on the zooplankton community should be high; small-bodied animals (<500 μ) will be eaten and numbers depressed and large-bodied forms (>500 μ) will not be eaten, though their numbers may be influenced indirectly by predation on neonates or removal of competitors. Cannibalism may act as a cyclopoid population regulator, since newborns are readily taken at high rates when naupliar density is high. In contrast to adults, 1st instar cyclopoid nauplii have little impact on other zooplankters. They do not predate, except on the smallest protozoa, and competition would be non-existent, since their food resources, dissolved organics and bacteria-coated detritus, are not

limiting (Wetzel, 1975). Survivorship studies of both adults and nauplii indicate unialgal and axenic bacterial cultures provide poor nourishment for long term survival, supporting the findings of Provasoli et al. (1959, 1970). Since aquatic systems have a rich variety of living and non-living foods, this finding has little application to natural populations; it is, however, an important consideration for in-laboratory research.

Comparison of methods. Three methods were used in the current study to detect predation: the prey difference method, predator gain method, and predator survivorship method. Each had strong points, weak points, and particular prey groups they worked with best.

One way to evaluate methods is to compare the results derived using different methods for feeding on a particular prey group. In the current study, those prey groups that were tested by more than one method were bacteria with adult cyclopoids (predator gain and survivorship methods) and bacteria and algae with the nauplius (prey difference and survivorship methods). Adult predation on bacteria was not detected by the predator gain method (*M. edax*), but was shown by the survivorship method (*C. vernalis*). Nauplii did not predate on bacteria nor algae according to the prey difference method (*C. b. thomasi*) nor the survivorship method (*C. vernalis*). The lack of agreement between adult predator gain and survivorship methods

is not surprising; the predator gain method lacked sensitivity while survivorship differences were slight (14.4 vs 12.1).

The prey difference method worked well for detecting predation on large prey (cladocera, nauplii, rotifers, paramecia), as counts of the entire feeding chamber could be made. This method worked poorly for small prey (bacteria, algae), since subsampling was required, introducing variability.

The survivorship method worked well for both adults and nauplii. All prey types were readily tested plus results other than mortality, such as egg production, molting, and observations on activity could be obtained. It is a tedious procedure requiring frequent observations and transfers. These transfers must be conducted carefully, to avoid losses and trauma to the animals.

The predator gain method was used during the summer of 1978 to detect cyclopoid feeding on the bacterium, Aeromonas, tagged with ^{32}P. Adult Mesocyclops edax were used with the goal of testing the nauplius later. Though the feeding experiment was unsuccessful and not repeated, the problems encountered do shed light on potential utility of the method for feeding experiments.

Aeromonas was labeled by growing stock cultures for 12 hours in nutrient broth, spiked with ^{32}P. This isotope was obtained as $H_2PO_4^-$ in 10 µCi amounts from American

Nuclear Products (Springfield, MO). All measurements of activity were done with a Geiger-Mueller Counter (Nuclear Chicago, Model 151A), using planchets as sample holders. Corrections were made and a standard curve was derived according to Chase and Rabinowitz (1967). Pilot studies with ^{32}P labeled algae and bacteria revealed that a Geiger-Mueller counter could be used to count radioactive cells and copepods without problems of self absorption.

Feeding trials with radioactive cells consisted of five steps: (1) introduction of the predator to a known quantity (number and activity) of cells, (2) the feeding period, (3) removal of the predator with a 240 μ filter and counting (number and activity), (4) removal of cells with a 0.45 μ filter and counting activity, and (5) precipitation of soluble ^{32}P as ammonium molyodiphosphate (Willard and Diehl, 1943) and counting activity. To check for gross error, a comparison was made between the counts introduced (specific activity X volume) and the total amount detected after the feeding trial.

Two controls were used. For the first, dead adult M. edax were placed in the tagged food suspension, which indicated the amount of radioactivity adsorbed passively. The second control consisted of live predators in contact with the filtrate (0.45 μ Millipore) from the labeled food suspension. This control detected the amount of soluble ^{32}P absorbed or swallowed by the predator.

The counts detected in the predator fractions were then compared with the counts in each control. For the current experiment (Table 4), graphic methods revealed no difference between experimental and control groups for CPM/predator. Therefore, significance was not tested statistically, nor were the number of cells ingested calculated.

The negative results obtained could have been due to the low specific activity of the bacteria (2 CPM/10^6 cells), requiring that a tremendous number of cells be ingested to detect feeding. This low specific activity was partly due to abundant (1.15%) phosphorous in the bacterial growth media. In this situation, added ^{32}P must compete with large amounts of the non-radioactive isotope for uptake by the cells. It would be more efficient to limit the concentration of phosphorus in the medium before adding the cells or the tracer. Phosphorus limitation can be readily achieved in algal growth media where all the nutrients are individually added but phorphorus limitation of bacterial growth media is difficult. The media comes in a complex form, with much of the phosphorus in organic form. Treatments for removing the phosphorus, such as precipitation as ammonium molybdiphosphate (Willard and Diehl, 1943), destroy the integrity of the medium, making it unsuitable for later bacterial growth.

Because of the complexity plus the hazards involved,

the radioisotope method is not recommended for most feeding studies. But it must be used for measuring feeding rates on algae, bacteria, or specific organic molecules since other methods are inadequate. The more sensitive liquid scintillation technique should be employed instead of the Geiger-Mueller counter. I suggest growing autotrophic rather than heterotrophic bacteria, so that a nutrient limited growth media can be obtained, insuring efficient uptake of the isotope.

SUMMARY

Feeding studies of adult carnivorous cyclopoids reveal:

1. Bacteria alone are not an adequate food source for long term survival.
2. Adults do not feed on algae.
3. Ciliated protozoa are readily taken; mixed protozoan cultures are a good food source for long term survival and fecundity.
4. Asplanchna are readily captured and eaten.
5. Cyclopoids predate on Bosmina and the prey are removed over time.
6. Cyclopoids attack, but fail to kill adult Ceriodaphnia.
7. Adults do not predate on ostracods.
8. Cyclopoids predate on their young (NI instar) and the removal rate is high.

Feeding studies of the first naupliar stage of carnivorous cyclopoids reveal:

1. Newborn nauplii do not feed on Ceriodaphnia, Bosmina, Asplanchna, nor Paramecium.

2. Feeding on algae and feeding on bacteria alone were not demonstrated. Prey difference results were unclear, but survivorship studies showed that neither food alone was adequate.

3. Nauplii do feed on bacteria adsorbed to inert particles (2-20 μ), but the amount of nutrition gained is slight.

4. Nauplii fed on dissolved organic matter (0.45 μ filtrate). They also utilized bacterial and/or protozoal populations that developed in the less pure vessels of this experiment.

Recommendations for future study.

1. Continue the naupliar survivorship studies.

   (a) Test algae that are known food sources, such as Scenedesmus, using the following combinations: axenic culture only, axenic culture plus protozoan culture filtrate, axenic culture plus one species of bacteria, two types of algae.

   (b) Test bacteria adsorbed to diatomaceous earth after separating the particles into size fractions (2-5 μ, 6-10 μ, etc.). Check a sample of these suspensions using the scanning electron microscope to verify attachment of bacterial cells to the particles.

   (c) Test protozoan culture filtrate as a food source with Scenedesmus added after the following lag times: 0 days, 2 days, 4 days, and 6 days. Dead nauplii should be examined and instar recorded.

   (d) Test the influence of various pesticides on naupliar survival.

2. Using the predator gain method, test the feeding rate of stages NI-NII, NIII-NV, NVI-CIII, and CIV-adult, on the following foods: bacteria alone, bacteria adsorbed to diatomaceous earth, 2 species of algae (1 motile and 1 non-motile), flagellates, and small ciliates.

3. Using adult cyclopoids and the prey difference method, test feeding rates on proven prey (such as Paramecium, Asplanchna, Bosmina, or cyclopoid nauplii) with various predator densities. Do cyclopoids interfere with one another's feeding?

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APPENDIX

Table 11. Calculations estimating the bacterial food requirements for maintaining the respiratory needs of mature *Mesocyclops edax*.

Constants	Reference
length of *Mesocyclops edax* = 1.5 mm	a
length of *Calanus* = 2.75 mm	b
respiratory rate of 1 *Calanus* = 0.6 µl O_2/h	b
energy equivalent of oxygen = 4.82×10^{-3} cal/µl O_2	c
weight of 1 bacterium = 2×10^{-13} g	d
caloric equivalent of bacteria = 5.5×10^{3} cal/g	e

Calculations

ratio of volume *M. edax*:volume *Calanus*

$$(1.5/2.75)^3 = 0.16$$

energy requirements of 1 *M. edax*

$$(0.16)(0.6\ \mu l\ O_2/h)(4.82 \times 10^{-3}\ cal/\mu l\ O_2)(24\ h/d) = 1.1 \times 10^{-2}\ cal/d$$

caloric value of 1 bacterium

$$(2 \times 10^{-13}\ g/cell)(5.5 \times 10^{3}\ cal/g) = 1.1 \times 10^{-9}\ cal/cell$$

required intake of bacteria

$$(1.1 \times 10^{-2}\ cal/d)/(1.1 \times 10^{-9}\ cal/cell) = 1.0 \times 10^{7}\ cells/d$$

References

a = CURRENT STUDY; b = Marshall (1973); c = Guyton (1976); d = Baker and Bradnum (1976); e = Prochazka, et al. (1973).

Table 12. Raw data from feeding trials.

Prey difference. Experiments are numbered in chronological order. For each experiment: Line one specifies the predator X prey; line two the date of experiment, contact time between predator and prey (h), and number of replicates; line three symbols represent control initial count (ci), control final count (cf), experimental initial count (ei), and experimental final count (ef); each subsequent line represents one replicate of control and experimental data. This data is summarized in Table 2.

1. C. b. thomasi adults X C. b. thomasi nauplii
   6/28/77, t = 20, n = 5

ci	cf	ei	ef
21	19	19	8
20	19	19	11
20	20	21	8
19	18	19	1
21	18	20	7

2. C. b. thomasi adults X Ceriodaphnia
   7/4/77, t = 27, n = 8

ci	cf	ei	ef
10	11	10	11
10	14	10	14
10	10	10	10
10	16	10	11
10	15	10	10
11	7	10	5
8	8	8	3
9	8	9	5

3. C. b. thomasi nauplii X Ceriodaphnia
   7/4/77, t = 27, n = 8, 5

ci	cf	ei	ef
10	11	8	8
10	14	10	8
10	10	9	7
10	16	10	8
10	15	8	6
11	7		
8	8		
9	8		

Table 12 (continued)

4. C. b. thomasi adults X ostracods
7/19/77, t = 24, n = 5

ci	cf	ei	ef
10	10	10	10
10	10	10	9
10	10	10	10
10	10	10	8
10	10	10	9

5. C. b. thomasi adults X Paramecium
7/28/77, t = 3, n = 5

ci	cf	ei	ef
70	61	70	32
70	66	70	47
70	66	70	45
70	65	70	36
70	74	70	48

6. C. b. thomasi nauplii X Paramecium
7/28/77, t = 3, n = 5

ci	cf	ei	ef
70	61	70	74
70	66	70	80
70	66	70	67
70	65	70	67
70	74	70	81

7. C. b. thomasi nauplii X unidentified algae
8/13/77, t = 6, n = 5, 10
(1st 5 experimental replicates had 1 nauplius per vessel; last 5 had 5 nauplii per vessel.)

ci	cf	ei	ef
948	347	--	531
506	501	--	511
570	444	--	621
469	359	--	391
524	359	--	708
			506
			439
			439
			356
			589

Table 12 (continued)

8. *C. b. thomasi* nauplii X *Enterobacter cloacae*
8/24/77, t = 4, n = 5

ci	cf	ei	ef
--	670	--	910
--	870	--	725
--	790	--	715
--	920	--	940
--	860	--	1505

9. *C. vernalis* adults X *Bosmina*
6/20/79, t = 48, n = 5

ci	cf	ei	ef
20	6	20	0
20	8	20	1
20	6	20	0
20	4	20	1
20	4	20	4

10. *C. vernalis* nauplii X *Bosmina*
6/20/79, t = 48, n = 5

ci	cf	ei	ef
20	6	20	4
20	8	20	4
20	6	20	4
20	4	20	3
20	4	20	7

11. *C. vernalis* adults X *Bosmina*
6/29/79, t = 12, n = 5

ci	cf	ei	ef
20	20	20	13
20	21	20	15
20	20	20	16
20	19	20	17
20	15	20	21

12. *C. vernalis* nauplii X *Bosmina*
6/29/79, t = 12, n = 5

ci	cf	ei	ef
20	20	20	14
20	21	20	20
20	20	20	19
20	19	20	20
20	15	20	21

Table 12 (continued)

13. C. vernalis adults X Asplanchna
6/29/79, t = 12, n = 5

ci	cf	ei	ef
20	27	20	22
20	22	20	16
20	28	20	23
20	34	20	21
20	29	20	17

14. C. vernalis nauplii X Asplanchna
6/29/79, t = 12, n = 5

ci	cf	ei	ef
20	27	20	31
20	22	20	29
20	28	20	24
20	34	20	30
20	29	20	23

Predator gain. Corrected counts per minute as a function of the number of M. edax (adults and copepodids) on filters. The copepods were counted after 12 hours contact with radiolabeled Aeromonas (9.4×10^7 cells/ml).

Experimental		Control #1 (dead pred)		Control #2 (filtrate)	
#	CPM	#	CPM	#	CPM
94	21	140	44	29	52
86	32	212	62	42	51
70	75	288	28	13	18

Table 12 (continued)

Predator survivorships. For each predator, the number of survivors are indicated by food type (X axis) and days from the start (Y axis). Food types tested were: starvation control (-), optimum food (+), bacteria (b), bacteria plus diatomaceous earth (bde), algae (a), protozoan culture filtrate (pcf), and protozoan culture filtrate with the predator washed 1, 2, 3, and 4 times.

1. C. vernalis nauplii

d	-	+	b	bde	a	pcf	1	2	3	4
0	54	45	54	9	18	18	18	18	18	18
1	54	45	53	9	18	18	18	18	18	18
2	53	45	53	9	13	17	17	18	17	17
3	47	45	50	9	10	16	15	17	16	17
4	39	45	41	9	7	16	15	17	16	16
5	13	44	15	7	4	15	12	15	16	16
6	2	42	4	3	4	14	10	9	6	11
7	1	40	1	1	2	11	1	6	3	6
8	0	35	1	0	2	10	0	1	0	1
9		32	1		0	8		1		0
10		30	0			8		0		
11		28				6				
12		27				4				
13		25				4				
14		25				1				
15		25				0				
16		24								
17		24								
18		23								
19		23								
20		22								
21		22								
22		21								
23		20								
24		19								
25		19								
26		19								
27		18								
28		15								

Table 12 (continued)

2. C. vernalis adults

d	-	+	b	a	pcf
0	10	5	10	5	5
1	10	5	10	5	5
2	10	5	10	5	5
3	10	5	10	5	5
4	10	5	10	5	5
5	10	5	10	4	4
6	10	5	10	4	4
7	10	5	10	4	4
8	10	5	10	4	4
9	9	5	10	3	4
10	9	5	10	3	4
11	9	5	10	3	3
12	9	5	10	2	3
13	5	5	10	2	2
14	2	5	6	2	1
15	1	5	2	2	0
16	1	5	2	2	
17	1	5	2	2	
18	0	5	1	0	
19		5	1		
20		5	1		
21		5	0		
22		5			
23		5			
24		5			
25		5			
26		5			
27		5			
28		5			
29		5			
30		5			
31		5			
32		5			
33		5			
34		5			
35		5			